

Atg2A siRNA (h): sc-96345

BACKGROUND

Atg2A (TG2 autophagy related 2 homolog A) is a 1,938 amino acid protein that belongs to the ATG2 family and may play a role in vesicle assembly. Encoded by a gene that maps to human chromosome 11q13.1, Atg2A is conserved in chimpanzee, canine, bovine, mouse and rat, and exists as four alternatively spliced isoforms. Undetected in adult tissues, including heart, brain, placenta, lung, liver and skeletal muscle, Atg2A regulation may act as a distinct indicator of autophagic programmed cell death. Atg2A is upregulated in both etoposide- and doxorubicin-induced apoptosis of HeLa cells, suggesting that Atg2A functions as a novel biomarker of topoisomerase II inhibitor-mediated apoptosis. Atg2A associates with Atg2B, indicating that these two related proteins also functionally interact. Atg2A frameshift mutations are linked to gastric and colorectal carcinomas with high microsatellite instability and may contribute to cancer development by deregulating the autophagy process.

REFERENCES

1. Van't Hof, A.E., et al. 2007. Evolutionary dynamics of multilocus microsatellite arrangements in the genome of the butterfly *Bicyclus anynana*, with implications for other lepidoptera. *Heredity* 98: 320-328.
2. Meléndez, A., et al. 2008. The cell biology of autophagy in metazoans: a developing story. *Development* 135: 2347-2360.
3. Kusama, Y., et al. 2009. Comprehensive analysis of expression pattern and promoter regulation of human autophagy-related genes. *Apoptosis* 14: 1165-1175.

CHROMOSOMAL LOCATION

Genetic locus: ATG2A (human) mapping to 11q13.1.

PRODUCT

Atg2A siRNA (h) 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 Atg2A shRNA Plasmid (h): sc-96345-SH and Atg2A shRNA (h) Lentiviral Particles: sc-96345-V as alternate gene silencing products.

For independent verification of Atg2A (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-96345A, sc-96345B and sc-96345C.

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

Atg2A siRNA (h) is recommended for the inhibition of Atg2A 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 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

Atg2A (G-1): sc-514207 is recommended as a control antibody for monitoring of Atg2A 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 Atg2A gene expression knockdown using RT-PCR Primer: Atg2A (h)-PR: sc-96345-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. Pfisterer, S.G., et al. 2014. Lipid droplet and early autophagosomal membrane targeting of Atg2A and Atg14L in human tumor cells. *J. Lipid Res.* 55: 1267-1278.
2. Bakula, D., et al. 2017. WIPI3 and WIPI4 β -propellers are scaffolds for LKB1-AMPK-TSC signalling circuits in the control of autophagy. *Nat. Commun.* 8: 15637.
3. Rosenthal, C.K. 2017. EGFR probes matrix stiffness. *Nat. Cell Biol.* 19: 600.

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.