SANTA CRUZ BIOTECHNOLOGY, INC.

Atg4C siRNA (h): sc-78773

BACKGROUND

Atg4C (ATG4 autophagy related 4 homolog C), also known as AUTL1 (AUT-like 1, cysteine endopeptidase), AUTL3 (AUT-like 3 cysteine endopeptidase), APG4-C, autophagin-3 or cysteine protease ATG4C, is a 458 amino acid tumor suppressor protein belonging to the peptidase C54 family. Encoded by a gene that maps to human chromosome 1p31.3, Atg4C is conserved in chimpanzee, dog, cow, mouse, rat, chicken and zebrafish. Atg4C is highly expressed in skeletal muscle, heart, liver and testis, and localizes to cytoplasm. Functioning as a cysteine protease, which is necessary for autophagy, Atg4C cleaves the C-terminal region of either MAP1LC3, GABARAPL2 or GABARAP. Atg4C is also inhibited by N-ethylmaleimide.

REFERENCES

1. Mariño, G., et al. 2007. Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3. J. Biol. Chem. 282: 18573-18583.
2. Apel, A., et al. 2008. Blocked autophagy sensitizes resistant carcinoma cells to radiation therapy. Cancer Res. 68: 1485-1494.
3. Morselli, E., et al. 2008. Mutant p53 protein localized in the cytoplasm inhibits autophagy. Cell Cycle 7: 3056-3061.
4. Fujita, N., et al. 2008. An Atg4B mutant hampers the lipidation of LC3 paralogues and causes defects in autophagosome closure. Mol. Biol. Cell 19: 4651-4659.
5. 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: ATG4C (human) mapping to 1p31.3.

PRODUCT

Atg4C 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Atg4C shRNA Plasmid (h): sc-78773-SH and Atg4C shRNA (h) Lentiviral Particles: sc-78773-V as alternate gene silencing products.

For independent verification of Atg4C (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-78773A, sc-78773B and sc-78773C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Atg4C siRNA (h) is recommended for the inhibition of Atg4C 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 µM in 66 µl. 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

Atg4C (287CT12.2.2): sc-517311 is recommended as a control antibody for monitoring of Atg4C 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Atg4C gene expression knockdown using RT-PCR Primer: Atg4C (h)-PR: sc-78773-PR (20 µl, 404 bp). 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.

PROTOCOLS

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