SANTA CRUZ BIOTECHNOLOGY, INC.

APG5 shRNA Plasmid (h): sc-41445-SH

BACKGROUND

In yeast, autophagy is an essential process for survival during nutrient starvation and cell differentiation. The process of autophagy is characterized as a non-selective degradation of cytoplasmic proteins into membrane stuctures called autophagosomes, and it is dependent on several proteins, including the autophagy proteins APG5 and APG7. Yeast Apg7 and the human homolog, APG7, share similarities with the ubiquitin-activating enzyme E1 in *Saccharomyces cerevisiae* and are likewise responsible for enzymatically activating the autophagy conjugation system. Apg5 and the human homolog, APG5 (also designated apoptosis-specific protein or APS), function as substrates for the autophagy protein Apg12. These proteins are covalently bonded together to form APG12/APG5 conjugates, which are required for the progression of autophagy.

REFERENCES

1. Kametaka, S., et al. 1996. Structural and functional analyses of APG5, a gene involved in autophagy in yeast. Gene 178: 139-143.
2. Klionsky, D.J. 1998. Nonclassical protein sorting to the yeast vacuole. J. Biol. Chem. 273: 10807-10810.

CHROMOSOMAL LOCATION

Genetic locus: ATG5 (human) mapping to 6q21.

PRODUCT

APG5 shRNA Plasmid (h) is a pool of 3 target-specific lentiviral vector plasmids each encoding 19-25 nt (plus hairpin) shRNAs designed to knock down gene expression. Each plasmid contains a puromycin resistance gene for the selection of cells stably expressing shRNA. Each vial contains 20 µg of lyophilized shRNA plasmid DNA. Suitable for up to 20 transfections. Also see APG5 siRNA (h): sc-41445 and APG5 shRNA (h) Lentiviral Particles: sc-41445-V as alternate gene silencing products.

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

APG5 shRNA Plasmid (h) is recommended for the inhibition of APG5 expression in human cells.

SUPPORT REAGENTS

For optimal shRNA Plasmid transfection efficiency, Santa Cruz Biotechnology's shRNA Plasmid Transfection Reagent: sc-108061 (0.2 ml) and shRNA Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control shRNAs are available as 20 µg lyophilized plasmid DNA. Each encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. Control shRNA Plasmids include: sc-108060, sc-108065 and sc-108066.

GENE EXPRESSION MONITORING

APG5 (C-1): sc-133158 is recommended as a control antibody for monitoring of APG5 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 APG5 gene expression knockdown using RT-PCR Primer: APG5 (h)-PR: sc-41445-PR (20 µl, 545 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Botta, G., et al. 2012. Inhibition of autophagy enhances the effects of E1A-defective oncolytic adenovirus dl922-947 against glioma cells *in vitro* and *in vivo*. Hum. Gene Ther. 23: 623-634.
2. Kim, T.S., et al. 2017. Ohmyungsamycins promote antimicrobial responses through autophagy activation via AMP-activated protein kinase pathway. Sci. Rep. 7: 3431.
3. Lee, Y.A., et al. 2018. Autophagy is a gatekeeper of hepatic differentiation and carcinogenesis by controlling the degradation of Yap. Nat. Commun. 9: 4962.
4. Sun, T., et al. 2021. Autophagy-mediated negative feedback attenuates the oncogenic activity of YAP in pancreatic cancer. Int. J. Biol. Sci. 17: 3634-3645.

PROTOCOLS

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