SANTA CRUZ BIOTECHNOLOGY, INC.

APG5 shRNA (h) Lentiviral Particles: sc-41445-V

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.

CHROMOSOMAL LOCATION

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

PRODUCT

APG5 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see APG5 siRNA (h): sc-41445 and APG5 shRNA Plasmid (h): sc-41445-SH as alternate gene silencing products.

APPLICATIONS

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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).

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.

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. Cufí, S., et al. 2011. Autophagy positively regulates the CD44+ CD24$^{-/low}$ breast cancer stem-like phenotype. Cell Cycle 10: 3871-3885.
2. Stankov, M.V., et al. 2012. Autophagy inhibition due to thymidine analogues as novel mechanism leading to hepatocyte dysfunction and lipid accumulation. AIDS 26: 1995-2006.
3. Yoon, J.S., et al. 2015. Autophagy is involved in the initiation and progression of Graves' orbitopathy. Thyroid 25: 445-454.
4. Guan, F., et al. 2016. Curcumin suppresses proliferation and migration of MDA-MB-231 breast cancer cells through autophagy-dependent Akt degradation. PLoS ONE 11: e0146553.
5. Carew, J.S., et al. 2017. Disruption of autophagic degradation with ROC-325 antagonizes renal cell carcinoma pathogenesis. Clin. Cancer Res. 23: 2869-2879.
6. Ye, T., et al. 2018. Oncolytic Newcastle disease virus induces autophagy-dependent immunogenic cell death in lung cancer cells. Am. J. Cancer Res. 8: 1514-1527.
7. Qureshi-Baig, K., et al. 2020. Hypoxia-induced autophagy drives colorectal cancer initiation and progression by activating the PRKC/PKC-EZR (ezrin) pathway. Autophagy 16: 1436-1452.
8. López-Valero, I., et al. 2020. Midkine signaling maintains the self-renewal and tumorigenic capacity of glioma initiating cells. Theranostics 10: 5120-5136.
9. Ilnytska, O., et al. 2021. Enrichment of NPC1-deficient cells with the lipid LBPA stimulates autophagy, improves lysosomal function, and reduces cholesterol storage. J. Biol. Chem. 297: 100813.
10. Miyakawa, K., et al. 2022. Galectin-9 restricts hepatitis B virus replication via p62/SQSTM1-mediated selective autophagy of viral core proteins. Nat. Commun. 13: 531.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.