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BECN1 shRNA (h2) Lentiviral Particles: sc-44286-V

BACKGROUND

BECN1 (Beclin 1) is a coiled-coil protein that has been implicated as an inhibitor of tumorigenesis. BECN1, which associates with Bcl-2, plays a significant role in autophagy. Autophagy is the degradation of cellular proteins in the lysosomes, and when this pathway is suppressed, cell growth is deregulated. Autophagy is controlled by the same signal transduction pathway that induces the phosphorylation of the Ribosomal Protein S6, and both are mediated via amino acids. BECN1 expression in various carcinoma cell lines such as MCF7 is low, whereas it is ubiquitously expressed in normal breast tissue. In transfected MCF7 cells, BECN1 complements autophagocytosis and, subsequently, inhibits cellular proliferation. Additionally, BECN1 shares structural similarity to the yeast autophagy gene product, Apg6, and was one of the first mammalian proteins discovered to mediate autophagy.

REFERENCES

1. Kisen, G.O., et al. 1993. Reduced autophagic activity in primary rat hepatocellular carcinoma and ascites hepatoma cells. *Carcinogenesis* 14: 2501-2505.
2. Blommaart, E.F., et al. 1995. Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes. *J. Biol. Chem.* 270: 2320-2326.
3. Blommaart, E.F., et al. 1997. Autophagic proteolysis: control and specificity. *Histochem. J.* 29: 365-385.
4. Liang, X.H., et al. 1998. Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2 interacting protein. *J. Virol.* 72: 8586-8596.
5. Liang, X.H., et al. 1999. Induction of autophagy and inhibition of tumorigenesis of beclin 1. *Nature* 402: 672-676.

CHROMOSOMAL LOCATION

Genetic locus: BECN1 (human) mapping to 17q21.31.

PRODUCT

BECN1 shRNA (h2) 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 μ l frozen stock containing 1.0×10^6 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 BECN1 siRNA (h2): sc-44286 and BECN1 shRNA Plasmid (h2): sc-44286-SH as alternate gene silencing products.

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.

PROTOCOLS

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

APPLICATIONS

BECN1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of BECN1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

BECN1 (H-300): sc-11427 is recommended as a control antibody for monitoring of BECN1 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor BECN1 gene expression knockdown using RT-PCR Primer: BECN1 (h2)-PR: sc-44286-PR (20 μ l, 458 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

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.

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.