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RNase L shRNA (h) Lentiviral Particles: sc-45965-V

BACKGROUND

RNase L encodes a component of the interferon-regulated 2-5A system that functions in the antiviral and antiproliferative roles of interferons. Mutations in this gene have been associated with predisposition to prostate cancer and this gene is a candidate for the hereditary prostate cancer 1 (HPC-1) allele. Interferon treatment enhances levels of both RNase L and a group of synthetases that produce 5'-triphosphorylated, 2',5'-oligoadenylates (2-5A) from ATP. The role of the 2-5A system in the control of viral and cellular growth suggests that defects in the 2-5A-dependent RNase gene could result in reduced immunity to virus infections and cancer.

REFERENCES

1. Wu, H., et al. 1998. Molecular cloning and expression of cDNA for human RNase H. *Antisense Nucleic Acid Drug Dev.* 8: 53-61.
2. Cerritelli, S., et al. 1998. Cloning, expression, and mapping of ribonucleases H of human and mouse related to bacterial RNase H1. *Genomics* 53: 300-307.
3. Demette, E., et al. 2002. Ribonuclease L proteolysis in peripheral blood mononuclear cells of chronic fatigue syndrome patients. *J. Biol. Chem.* 277: 35746-35751.
4. ten Asbroek, A., et al. 2002. Ribonuclease H1 maps to chromosome 2 and has at least three pseudogene loci in the human genome. *Genomics* 79: 818-23.
5. Nakazato, H., et al. 2003. Role of genetic polymorphisms of the RNase L gene on familial prostate cancer risk in a Japanese population. *Br. J. Cancer* 89: 691-696.
6. Silverman, R.H. 2003. Implications for RNase L in prostate cancer biology. *Biochemistry* 42: 1805-1812.

CHROMOSOMAL LOCATION

Genetic locus: RNASEL (human) mapping to 1q25.3.

PRODUCT

RNase L 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 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 RNase L siRNA (h): sc-45965 and RNase L shRNA Plasmid (h): sc-45965-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

RNase L shRNA (h) Lentiviral Particles is recommended for the inhibition of RNase L 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

RNase L (E-9): sc-74405 is recommended as a control antibody for monitoring of RNase L 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor RNase L gene expression knockdown using RT-PCR Primer: RNase L (h)-PR: sc-45965-PR (20 μ l, 537 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.