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RNase III Drosha shRNA (h) Lentiviral Particles: sc-44080-V

BACKGROUND

The ribonuclease III superfamily represents a structurally distinct group of double-strand-specific endonucleases with essential roles in RNA maturation, RNA decay and gene silencing. Initial cleavage of microRNAs is catalysed by Drosha, a nuclease of the RNase III family, which acts on primary miRNA transcripts (pri-miRNAs) in the nucleus. Human Drosha is a component of two multi-protein complexes. The larger complex contains multiple classes of RNA-associated proteins including RNA helicases, proteins that bind double-stranded RNA, novel heterogeneous nuclear ribonucleoproteins and the Ewing's sarcoma family of proteins. The smaller complex is composed of Drosha and the double-stranded-RNA-binding protein, DGCR8.

REFERENCES

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- Gregory R.I., et al. 2004. The Microprocessor complex mediates the genesis of microRNAs. *Nature* 432: 235-240.
- Sun W., et al. 2004. Mutational analysis of the nuclease domain of *Escherichia coli* ribonuclease III. Identification of conserved acidic residues that are important for catalytic function *in vitro*. *Biochem.* 43: 13054-13062.
- Han, J., et al. 2004. The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev.* 18: 3016-3027.
- Landthaler, M., et al. 2004. The human DiGeorge syndrome critical region gene 8 and its *Drosophila melanogaster* homolog are required for miRNA biogenesis. *Curr. Biol.* 14: 2162-2167.
- Tomari, Y., et al. 2005. MicroRNA biogenesis: Drosha can't cut it without a partner. *Curr. Biol* 15: R61-R64.

CHROMOSOMAL LOCATION

Genetic locus: RNASEN (human) mapping to 5p13.3.

PRODUCT

RNase III Drosha 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 III Drosha siRNA (h): sc-44080 and RNase III Drosha shRNA Plasmid (h): sc-44080-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 III Drosha shRNA (h) Lentiviral Particles is recommended for the inhibition of RNase III 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 III Drosha (C-7): sc-393591 is recommended as a control antibody for monitoring of RNase III Drosha 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 III gene expression knockdown using RT-PCR Primer: RNase III Drosha (h)-PR: sc-44080-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.