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DcpS shRNA (m) Lentiviral Particles: sc-44390-V

BACKGROUND

Eukaryotic cells primarily utilize exoribonucleases and decapping enzymes to degrade their mRNA. DcpS is a scavenger pyrophosphatase that hydrolyzes the residual cap structure following 3' to 5' decay of an mRNA. Following mRNA degradation DcpS releases N-7 methyl guanosine monophosphate and 5'-diphosphate terminated cap or mRNA products. The central histidine within the DcpS HIT motif is critical for decapping activity and defines the HIT motif as a new mRNA decapping domain, making DcpS the first member of the HIT family of proteins with a defined biological function. HIT proteins are homodimeric and contain two conserved 100-amino-acid HIT fold domains with independent active sites that are each sufficient to bind and hydrolyze cognate substrates.

REFERENCES

1. Fireman, P. 1992. Diagnosis of sinusitis in children: emphasis on the history and physical examination. *J. Allergy Clin. Immunol.* 90: 433-436.
2. Wang, Z., et al. 2001. Functional link between the mammalian exosome and mRNA decapping. *Cell* 107: 751-762.
3. Liu, H., et al. 2002. The scavenger mRNA decapping enzyme DcpS is a member of the HIT family of pyrophosphatases. *EMBO J.* 21: 4699-4708.
4. Wang, Z., et al. 2002. The hDcp2 protein is a mammalian mRNA decapping enzyme. *Proc. Natl. Acad. Sci. USA* 99: 12663-12668.
5. Gu, M., et al. 2004. Insights into the structure, mechanism, and regulation of scavenger mRNA decapping activity. *Mol. Cell* 14: 67-80.

CHROMOSOMAL LOCATION

Genetic locus: Dcps (mouse) mapping to 9 A4.

PRODUCT

DcpS shRNA (m) 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 DcpS siRNA (m): sc-44390 and DcpS shRNA Plasmid (m): sc-44390-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

DcpS shRNA (m) Lentiviral Particles is recommended for the inhibition of DcpS expression in mouse 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

DcpS (A-12): sc-393226 is recommended as a control antibody for monitoring of DcpS 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 DcpS gene expression knockdown using RT-PCR Primer: DcpS (m)-PR: sc-44390-PR (20 μ l). 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.