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DcpS siRNA (h): sc-44389

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 (human) mapping to 11q24.2.

PRODUCT

DcpS siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DcpS shRNA Plasmid (h): sc-44389-SH and DcpS shRNA (h) Lentiviral Particles: sc-44389-V as alternate gene silencing products.

For independent verification of DcpS (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44389A, sc-44389B and sc-44389C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

DcpS siRNA (h) is recommended for the inhibition of DcpS expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor DcpS gene expression knockdown using RT-PCR Primer: DcpS (h)-PR: sc-44389-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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