SANTA CRUZ BIOTECHNOLOGY, INC.

Dcp1a siRNA (m): sc-45780

BACKGROUND

Cleavage of the 5'-cap structure is involved in the major 5'-to-3' and nonsense-mediated mRNA decay pathways. The protein complex consisting of Dcp1 and Dcp2 has been identified as the species responsible for the decapping reaction in *Saccharomyces cerevisiae*. In nonsense-mediated decay, the human decapping complex, made up of *S. cerevisiae* homologs hDcp1a and hDcp2, may be recruited to mRNAs containing premature termination codons by nonsense-mediated decay factor (Upf) proteins. hDcp2 specifically hydrolyzes methylated capped RNA to release m(7)GDP, thereby aiding in mRNA degradation. Both hDcp1a and hDcp2 colocalize in the cytoplasm. In addition, hDcp1a interacts with Smad4 forming a complex with TGFβ and BMP-4. hDcp1a and Smad4 interact directly through a EVH1/WH1 domain on hDcp1a and a proline-rich activation domain on Smad4. Smad4 is essential to nuclear translocation of hDcp1a as deletion of the Smad4-interacting domain (located in the N-terminal 100 amino acids) of hDcp1a eliminates TGFβ-induced nuclear translocation of hDcp1a.

REFERENCES

1. LaGrandeur, T.E., et al. 1998. Isolation and characterization of Dcp1p, the yeast mRNA decapping enzyme. EMBO J. 17: 1487-1496.
2. Itoh, S., et al. 2000. Signaling of transforming growth factor β family members through Smad proteins. Eur. J. Biochem. 267: 6954-6967.
3. Tucker, M., et al. 2000. Mechanisms and control of mRNA decapping in *Saccharomyces cerevisiae*. Annu. Rev. Biochem. 69: 571-595.
4. Moustakas, A., et al. 2001. Smad regulation in TGFβ signal transduction. J. Cell Sci. 114: 4359-4369.
5. Callebaut, I. 2002. An EVH1/WH1 domain as a key actor in TGβ signalling. FEBS Lett. 519: 178-180.
6. Chen, W., et al. 2002. Review of current progress in the structure and function of Smad proteins. Chin. Med. J. 115: 446-450.
7. Bai, R.Y., et al. 2002. SMIF, a Smad4-interacting protein that functions as a co-activator in TGFβ signalling. Nat. Cell Biol. 4: 181-190.
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CHROMOSOMAL LOCATION

Genetic locus: Dcp1a (mouse) mapping to 14 B.

PRODUCT

Dcp1a siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Dcp1a shRNA Plasmid (m): sc-45780-SH and Dcp1a shRNA (m) Lentiviral Particles: sc-45780-V as alternate gene silencing products.

For independent verification of Dcp1a (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45780A, sc-45780B and sc-45780C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Dcp1a siRNA (m) is recommended for the inhibition of Dcp1a expression in mouse 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 µM in 66 µl. 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

Dcp1a (56-Y): sc-100706 is recommended as a control antibody for monitoring of Dcp1a 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Dcp1a gene expression knockdown using RT-PCR Primer: Dcp1a (m)-PR: sc-45780-PR (20 µl). 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.