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SANTA CRUZ BIOTECHNOLOGY, INC.

NDH II siRNA (m): sc-45707

BACKGROUND

Pre-mRNA splicing is a critical step in the posttranscriptional regulation of gene expression. Several protein complexes are involved in proper mRNA splicing and transport. The small nuclear ribonucleoprotein particles (snRNPs) interact with the SRm160/300 splicing coactivator complex to form a large RNA spliceosome. The heterogeneous nuclear ribonucleoproteins (hnRNPs) contribute to the processing and transport of pre-mRNA within the spliceosome. Also, the exon junction complex (EJC), which includes Y14, Aly/REF and Magoh, mediates mRNA export, cytoplasmic localization and nonsense-mediated mRNA decay. The effect on pre-mRNA splicing involves a nuclear complex (CBC). CBC consists of two cap binding proteins, CBP20 and CBP80, which mediate the stimulatory functions of the cap in pre-mRNA splicing, 3'-end formation and U snRNA export. Splicing factor 1 is a nuclear protein that binds the branch point sequence of pre-mRNA in the first step of spliceosome assembly and SRp55 modulates the selection of alternative splice sites in constitutive splicing. Nuclear DNA helicase II (NDH II), also known as RNA Helicase A, generates secondary structures that interact with RNA-binding proteins. MDA5 is an ATP-dependent RNA helicase associated with the growth, differentiation and death of human melanoma cells.

REFERENCES

1. Kang, D.C., et al. 2002. MDA5: an interferon-inducible putative RNA Helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties. Proc. Natl. Acad. Sci. USA 99: 637-642.
2. Zhang, S., et al. 2004. Multiple functions of nuclear DNA Helicase II (RNA Helicase A) in nucleic acid metabolism. Acta Biochim. Biophys. Sin. 36: 177-183.
3. Zhang, S., et al. 2004. Nuclear DNA Helicase II (RNA Helicase A) binds to an F-Actin containing shell that surrounds the nucleolus. Exp. Cell Res. 293: 248-258.
4. Zhang, S., et al. 2004. DNA-dependent protein kinase (DNA-PK) phosphorylates nuclear DNA helicase II/RNA Helicase A and hnRNP proteins in an RNA-dependent manner. Nucleic Acids Res. 32: 1-10.

CHROMOSOMAL LOCATION

Genetic locus: Dhx9 (mouse) mapping to 1 G3.

PRODUCT

NDH II 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 NDH II shRNA Plasmid (m): sc-45707-SH and NDH II shRNA (m) Lentiviral Particles: sc-45707-V as alternate gene silencing products.

For independent verification of NDH II (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-45707A, sc-45707B and sc-45707C.

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

NDH II siRNA (m) is recommended for the inhibition of NDH II 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

NDH II (B-9): sc-137232 is recommended as a control antibody for monitoring of NDH II 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 NDH II gene expression knockdown using RT-PCR Primer: NDH II (m)-PR: sc-45707-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.