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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

NDH II shRNA (h) Lentiviral Particles: sc-45706-V

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 (human) mapping to 1q25.3.

PRODUCT

NDH II 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 NDH II siRNA (h): sc-45706 and NDH II shRNA Plasmid (h): sc-45706-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.

APPLICATIONS

NDH II shRNA (h) Lentiviral Particles is recommended for the inhibition of NDH II 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

NDH II (R-16): sc-46422 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NDH II gene expression knockdown using RT-PCR Primer: NDH II (h)-PR: sc-45706-PR (20 μ l, 584 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

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