

NELF-D siRNA (h): sc-75898

BACKGROUND

NELF-D (negative elongation factor C/D), also known as TH1, NELF-C, HSPC130 or TH1L, is a 590 amino acid protein that localizes to the nucleus and exists as a component of the multi-protein NELF complex, a structure which negatively regulates Pol II-dependent transcription elongation. Expressed in a variety of tissues, including liver, heart, kidney, lung, brain, placenta and pancreas, NELF-D is involved in controlling transcriptional pausing of Pol II and may be able to induce chromatin unfolding, possibly playing a role in tumorigenesis. NELF-D is encoded by a gene which maps to human chromosome 20. Comprising approximately 2% of the human genome, chromosome 20 contains nearly 63 million bases that encode over 600 genes, some of which are associated with Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, spinal muscular atrophy, ring chromosome 20 epilepsy syndrome and Alagille syndrome. Additionally, chromosome 20 contains a region with numerous genes which are thought important for seminal production and may be potential targets for male contraception.

REFERENCES

1. Ping, Y.H. and Rana, T.M. 2001. DSIF and NELF interact with RNA polymerase II elongation complex and HIV-1 Tat stimulates P-TEFb-mediated phosphorylation of RNA polymerase II and DSIF during transcription elongation. *J. Biol. Chem.* 276: 12951-12958.
2. Narita, T., et al. 2003. Human transcription elongation factor NELF: identification of novel subunits and reconstitution of the functionally active complex. *Mol. Cell. Biol.* 23: 1863-1873.
3. Wu, C.H., et al. 2005. Molecular characterization of *Drosophila* NELF. *Nucleic Acids Res.* 33: 1269-1279.
4. Joó, J.G., et al. 2006. Trisomy 20 mosaicism and nonmosaic trisomy 20: a report of 2 cases. *J. Reprod. Med.* 51: 209-212.

CHROMOSOMAL LOCATION

Genetic locus: NELFCD (human) mapping to 20q13.32.

PRODUCT

NELF-D 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 NELF-D shRNA Plasmid (h): sc-75898-SH and NELF-D shRNA (h) Lentiviral Particles: sc-75898-V as alternate gene silencing products.

For independent verification of NELF-D (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-75898A, sc-75898B and sc-75898C.

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.

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

NELF-D siRNA (h) is recommended for the inhibition of NELF-D 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

NELF-D (C-10): sc-393972 is recommended as a control antibody for monitoring of NELF-D 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 NELF-D gene expression knockdown using RT-PCR Primer: NELF-D (h)-PR: sc-75898-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.