

NELF-A siRNA (m): sc-38096

BACKGROUND

NELF-A, for negative elongation factor A, is a protein factor required for DRB-sensitive transcription. NELF-A is one of the five components of the multisubunit NELF complex that cooperates with DSIF to repress RNA polymerase II elongation. Control of transcription elongation requires a complex interplay between positive transcription elongation factor b (P-TEFb) and negative transcription elongation factors, DSIF and NELF. DSIF and NELF act as negative transcription elongation factors by increasing the time the polymerase spends at pause sites. DSIF/NELF inhibition of transcription is prevented by P-TEFb in cooperation with FACT. NELF-A is also known as WHSC2 (Wolf-Hirschhorn syndrome candidate 2). Wolf-Hirschhorn syndrome is a multiple malformation syndrome characterized by mental and developmental defects resulting from a hemizygous deletion of the distal short arm of chromosome 4 (4p16.3). The human NELF-A gene maps to chromosome 4p16.3 and encodes a 528 amino acid protein that is expressed in endothelial cells.

REFERENCES

1. Yamaguchi, Y., et al. 1999. NELF, a multisubunit complex containing RD, cooperates with DSIF to repress RNA polymerase II elongation. *Cell* 97: 41-51.
2. Wright, T.J., et al. 1999. Comparative analysis of a novel gene from the Wolf-Hirschhorn/Pitt-Rogers-Danks syndrome critical region. *Genomics* 59: 203-212.
3. Mariotti, M., et al. 2000. Modulation of WHSC2 expression in human endothelial cells. *FEBS Lett.* 487: 166-170.
4. Wada, T., et al. 2000. FACT relieves DSIF/NELF-mediated inhibition of transcriptional elongation and reveals functional differences between P-TEFb and TFIIF. *Mol. Cell* 5: 1067-1072.
5. 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.
6. Renner, D.B., et al. 2001. A highly purified RNA polymerase II elongation control system. *J. Biol. Chem.* 276: 42601-42609.

CHROMOSOMAL LOCATION

Genetic locus: Whsc2 (mouse) mapping to 5 B2.

PRODUCT

NELF-A 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NELF-A siRNA Plasmid (m): sc-38096-SH and NELF-A shRNA (m) Lentiviral Particles: sc-38096-V as alternate gene silencing products.

For independent verification of NELF-A (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-38096A, sc-38096B and sc-38096C.

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-A siRNA (m) is recommended for the inhibition of NELF-A 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 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-A (G-11): sc-365004 is recommended as a control antibody for monitoring of NELF-A 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-A gene expression knockdown using RT-PCR Primer: NELF-A (m)-PR: sc-38096-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.