SANTA CRUZ BIOTECHNOLOGY, INC.

NELF-E siRNA (h): sc-38093

BACKGROUND

NELF-E, for negative elongation factor E, is a putative RNA binding protein. NELF-E 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-E is also known as RD and RDBP (RD RNA-binding protein). RD, the acronym of the most common dipeptide repeat describes the single letter symbols for arginine (R) and aspartic acid (D), respectively. NELF-E has a functional RNA-binding domain, whose mutations impair transcription repression without affecting known protein-protein interactions. The human NELF-E gene maps to chromosome 6p21.33 and encodes a 371 amino acid protein.

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. Online Mendelian Inheritance in Man, OMIM™. 1999. Johns Hopkins University, Baltimore, MD. MIM Number: 154040. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Wada, T., et al. 2000. FACT relieves DSIF/NELF-mediated inhibition of transcriptional elongation and reveals functional differences between P-TEFb and TFIIH. Mol. Cell 5: 1067-1072.
4. 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.
5. 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: NELFE (human) mapping to 6p21.33.

PRODUCT

NELF-E 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NELF-E shRNA Plasmid (h): sc-38093-SH and NELF-E shRNA (h) Lentiviral Particles: sc-38093-V as alternate gene silencing products.

For independent verification of NELF-E (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-38093A, sc-38093B and sc-38093C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

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

NELF-E (F-9): sc-377052 is recommended as a control antibody for monitoring of NELF-E 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 NELF-E gene expression knockdown using RT-PCR Primer: NELF-E (h)-PR: sc-38093-PR (20 µl, 587 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.