SANTA CRUZ BIOTECHNOLOGY, INC.

SPT5 siRNA (m): sc-38441

BACKGROUND

SPT4 (also designated Suppressor of Ty4 and p14) and SPT5 (also designated DSIF p160) are highly conserved proteins from yeast to humans. Nuclear SPT4 and SPT5 are involved in both DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole)-mediated transcriptional inhibition as well as the activation of transcriptional elongation by the HIV-1 protein Tat. SPT4 binds SPT5 to form the DSIF (DRB-sensitivity-inducing factor) complex, which binds RNA polymerase II and directly regulates elongation. However, SPT5 protein in mitotic HeLa cells migrates more slowly on SDS-PAGE than does SPT5 isolated from interphase cells, as a result of enhanced SPT5 phosphorylation. The C-terminal CTR1 domain of SPT5 is the substrate for P-TEFb phosphorylation, which is critical for SPT5 function as a regulator of transcriptional elongation.

REFERENCES

1. Chiang, P.W., et al. 1996. Isolation and characterization of the human and mouse homologues (SUPT4H and Supt4h) of the yeast SPT4 gene. Genomics 34: 368-375.
2. Hartzog, G.A., et al. 1996. Identification and analysis of a functional human homolog of the SPT4 gene of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 16: 2848-2856.
3. Wada, T., et al. 1998. Evidence that P-TEFb alleviates the negative effect of DSIF on RNA polymerase II-dependent transcription *in vitro*. EMBO J. 17: 7395-7403.
4. Wada, T., et al. 1998. DSIF, a novel transcription elongation factor that regulates RNA polymerase II processivity, is composed of human SPT4 and SPT5 homologs. Genes Dev. 12: 343-356.
5. Yamaguchi, Y., et al. 1999. Structure and function of the human transcription elongation factor DSIF. J. Biol. Chem. 274: 8085-8092.
6. Ivanov, D., et al. 2000. Domains in the SPT5 protein that modulate its transcriptional regulatory properties. Mol. Cell. Biol. 20: 2970-2983.
7. Xiao, Y., et al. 2005. Analysis of a splice array experiment elucidates roles of chromatin elongation factor SPT4-5 in splicing. PLoS Comput. Biol. 1: E39.
8. Reyes-Reyes, M., et al. 2007. Role for the Ssu72 C-terminal domain phosphatase in RNA polymerase II transcription elongation. Mol. Cell. Biol. 27: 926-936.

CHROMOSOMAL LOCATION

Genetic locus: Supt5h (mouse) mapping to 7 A3.

PRODUCT

SPT5 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 SPT5 shRNA Plasmid (m): sc-38441-SH and SPT5 shRNA (m) Lentiviral Particles: sc-38441-V as alternate gene silencing products.

For independent verification of SPT5 (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-38441A, sc-38441B and sc-38441C.

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

SPT5 siRNA (m) is recommended for the inhibition of SPT5 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

SPT5 (D-3): sc-133217 is recommended as a control antibody for monitoring of SPT5 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 SPT5 gene expression knockdown using RT-PCR Primer: SPT5 (m)-PR: sc-38441-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.