



SPT16 siRNA (m): sc-37876

BACKGROUND

Expression of protein-coding genes requires the association of specific transcription factors, RNA polymerase and various accessory factors. These accessory factors are distinguished as either histone acetyltransferases or ATP-dependent chromatin-remodeling enzymes, which include FACT (for facilitates chromatin transcription), and they facilitate transcription initiation on DNA packaged into chromatin. FACT is a chromatin-specific elongation factor required for transcription of chromatin templates, and it specifically interacts with nucleosomes and Histone H2A/H2B dimers to promote nucleosome disassembly upon transcription. FACT represents a complex between SPT16, a homolog of the *Saccharomyces cerevisiae* Spt16/Cdc68 protein, and the high-mobility group (HMG)-1-like protein structure-specific recognition protein-1 (SSRP-1). Similar to other HMG domain containing proteins, which are characterized by their ability to bend target DNAs, SSRP1 and the murine ortholog T160 physically interact with serum response factors (SRF) and function as a positive co-regulatory proteins involved in modulating SRF-dependent gene expression.

REFERENCES

1. Felsenfeld, G. 1992. Chromatin as an essential part of the transcriptional mechanism. *Nature* 355: 219-224.
2. Wittmeyer, J. and Formosa, T. 1997. The *Saccharomyces cerevisiae* DNA polymerase alpha catalytic subunit interacts with Cdc68/Spt16 and with Pob3, a protein similar to an HMG1-like protein. *Mol. Cell. Biol.* 17: 4178-4190.
3. Shilatfard, A. 1998. Factors regulating the transcriptional elongation activity of RNA polymerase II. *FASEB J.* 12: 1437-1446.
4. Orphanides, G., LeRoy, G., Chang, C.H., Luse, D.S. and Reinberg, D. 1998. FACT, a factor that facilitates transcript elongation through nucleosomes. *Cell* 92: 105-116.
5. LeRoy, G., Orphanides, G., Lane, W.S. and Reinberg, D. 1998. Requirement of RSF and FACT for transcription of chromatin templates *in vitro*. *Science* 282: 1900-1904.
6. Dyer, M.A., Hayes, P.J. and Baron, M.H. 1998. The HMG domain protein SSRP1/PREIIBF is involved in activation of the human embryonic β -like globin gene. *Mol. Cell. Biol.* 18: 2617-2628.

CHROMOSOMAL LOCATION

Genetic locus: Supt16h (mouse) mapping to 14 C2.

PRODUCT

SPT16 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 SPT16 shRNA Plasmid (m): sc-37876-SH and SPT16 shRNA (m) Lentiviral Particles: sc-37876-V as alternate gene silencing products.

For independent verification of SPT16 (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-37876A, sc-37876B and sc-37876C.

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

SPT16 siRNA (m) is recommended for the inhibition of SPT16 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

SPT16 (D-3): sc-377028 is recommended as a control antibody for monitoring of SPT16 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 SPT16 gene expression knockdown using RT-PCR Primer: SPT16 (m)-PR: sc-37876-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.