

RPA135 siRNA (h): sc-36436

BACKGROUND

RNA polymerases transcribe nuclear genes for ribosomal RNA, thus representing ribosomal biogenesis. RNA polymerase I (Pol I) is located in the nucleolus and transcribes class I genes, which code for large ribosomal RNA. Different subunits of the Pol I transcription machinery are targets of various physiological stimuli, which suggests that multiple signaling pathways are involved in carrying out Pol I transcription. RPA16, RPA40 and RPA135 are subunits of Pol I that associate with each other at an early stage of RNA Pol I assembly. RPA40 is essential for the function and integrity of the complex and is also an essential subunit of RNA polymerase III (Pol III).

REFERENCES

1. Nogi, Y., et al. 1991. An approach for isolation of mutants defective in 35S ribosomal RNA synthesis in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 16: 7026-7030.
2. Yao, Y., et al. 1996. Mouse RNA polymerase I 16 kDa subunit able to associate with 40 kDa subunit is a homolog of yeast AC19 subunit of RNA polymerases I and III. J. Biol. Chem. 51: 32881-32885.
3. Seither, P., et al. 1997. Molecular cloning and characterization of the cDNA encoding the largest subunit of mouse RNA polymerase I. Mol. Gen. Genet. 2: 180-186.
4. Hoeger, H., et al. 1998. Deficient transcription of subunit RPA 40 of RNA polymerase I and III in heart of rats with neonatal asphyxia. Life Sci. 4: 275-282.
5. Grummt, I. 1999. Regulation of mammalian ribosomal gene transcription by RNA polymerase I. Prog. Nucleic Acid Res. Mol. Biol. 62: 109-154.
6. Chen, H.K., et al. 1999. Human Nopp140, which interacts with RNA polymerase I: implications for rRNA gene transcription and nucleolar structural organization. Mol. Cell. Biol. 12: 8536-8546.
7. Mosgoeller, W., et al. 2000. Brain RNA polymerase and nucleolar structure in perinatal asphyxia of the rat. Exp. Neurol. 1: 174-182.

CHROMOSOMAL LOCATION

Genetic locus: POLR1B (human) mapping to 2q13.

PRODUCT

RPA135 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 RPA135 shRNA Plasmid (h): sc-36436-SH and RPA135 shRNA (h) Lentiviral Particles: sc-36436-V as alternate gene silencing products.

For independent verification of RPA135 (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-36436A, sc-36436B and sc-36436C.

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

RPA135 siRNA (h) is recommended for the inhibition of RPA135 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

RPA135 (4H6): sc-293272 is recommended as a control antibody for monitoring of RPA135 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 RPA135 gene expression knockdown using RT-PCR Primer: RPA135 (h)-PR: sc-36436-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.