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Pol III RPC32 siRNA (h): sc-43507

BACKGROUND

Eukaryotes produce three distinct classes of RNA polymerase, Pol I, II and III. Each polymerase is responsible for the synthesis of a different class of RNA. RNA polymerase I (Pol I) transcribes the rRNA (ribosomal RNA) genes for the precursor of the 28S, 18S and 5.8S molecules of the ribosome. RNA polymerase II transcribes protein-encoding genes into mRNA (messenger RNA) and snRNA (small nuclear RNA) genes into snRNAs that influence the processing of other classes of RNA. RNA polymerase III (Pol III) transcribes the 5S rRNA genes and all of the tRNA (transfer RNA) genes.

REFERENCES

1. Bushnell, D.A., et al. 2004. Structural basis of transcription: an RNA polymerase II-TFIIB cocrystal at 4.5 angstroms. *Science* 303: 983-988.
2. Palangat, M., et al. 2004. Downstream DNA selectively affects a paused conformation of human RNA polymerase II. *J. Mol. Biol.* 341: 429-442.
3. Zhong, S., et al. 2004. Epidermal growth factor enhances cellular TATA binding protein levels and induces RNA polymerase I- and III-dependent gene activity. *Mol. Cell. Biol.* 24: 5119-5129.
4. Hirsch, H.A., et al. 2004. Distinct mechanisms for repression of RNA polymerase III transcription by the retinoblastoma tumor suppressor protein. *Mol. Cell. Biol.* 24: 5989-5999.
5. White, R.J. 2004. RNA polymerase III transcription and cancer. *Oncogene* 23: 3208-3216.

CHROMOSOMAL LOCATION

Genetic locus: POLR3G (human) mapping to 5q14.3.

PRODUCT

Pol III RPC32 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 Pol III RPC32 shRNA Plasmid (h): sc-43507-SH and Pol III RPC32 shRNA (h) Lentiviral Particles: sc-43507-V as alternate gene silencing products.

For independent verification of Pol III RPC32 (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-43507A, sc-43507B and sc-43507C.

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

Pol III RPC32 siRNA (h) is recommended for the inhibition of Pol III RPC32 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

Pol III RPC32 (H-9): sc-48365 is recommended as a control antibody for monitoring of Pol III RPC32 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 Pol III RPC32 gene expression knockdown using RT-PCR Primer: Pol III RPC32 (h)-PR: sc-43507-PR (20 μ l, 542 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

SELECT PRODUCT CITATIONS

1. Veronese, A., et al. 2014. Allele-specific loss and transcription of the miR-15a/16-1 cluster in chronic lymphocytic leukemia. *Leukemia* 29: 86-95.

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.