SANTA CRUZ BIOTECHNOLOGY, INC.

POLR3E siRNA (h): sc-93337

BACKGROUND

Eukaryotes produce 3 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 III (Pol III) transcribes the 5S rRNA genes and all of the tRNA (transfer RNA) genes. POLR3E, also known as DNA-directed RNA polymerase III subunit RPC5, is a 708 amino acid nuclear protein that is one of the seventeen subunits that comprise Pol III. Specifically, POLR3E is a DNA-dependent RNA polymerase that uses the four ribonucleoside triphosphates to catalyze the transcription of DNA to RNA. The gene encoding POLR3K maps to human chromosome 16, which encodes over 900 genes in approximately 90 million base pairs and is associated with a variety of genetic disorders.

REFERENCES

1. Ittmann, M., et al. 1987. Isolation of the human gene that complements a temperature-sensitive cell cycle mutation in BHK cells. Mol. Cell. Biol. 7: 3386-3393.
2. Ittmann, M., et al. 1993. The gene complementing a temperature-sensitive cell cycle mutant of BHK cells is the human homologue of the yeast RPC53 gene, which encodes a subunit of RNA polymerase C (III). Cell Growth Differ. 4: 503-511.
3. Ittmann, M.M. 1994. Cell cycle control of the BN51 cell cycle gene which encodes a subunit of RNA polymerase III. Cell Growth Differ. 5: 783-788.
4. Jackson, A.J., et al. 1995. The BN51 protein is a polymerase (Pol)-specific subunit of RNA Pol III which reveals a link between Pol III transcription and pre-rRNA processing. Mol. Cell. Biol. 15: 94-101.
5. Chong, S.S., et al. 2001. Reconstitution of transcription from the human U6 small nuclear RNA promoter with eight recombinant polypeptides and a partially purified RNA polymerase III complex. J. Biol. Chem. 276: 20727-20734.
6. Hu, P., et al. 2002. Characterization of human RNA polymerase III identifies orthologues for Saccharomyces cerevisiae RNA polymerase III subunits. Mol. Cell. Biol. 22: 8044-8055.

CHROMOSOMAL LOCATION

Genetic locus: POLR3E (human) mapping to 16p12.2.

PRODUCT

POLR3E 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 POLR3E shRNA Plasmid (h): sc-93337-SH and POLR3E shRNA (h) Lentiviral Particles: sc-93337-V as alternate gene silencing products.

For independent verification of POLR3E (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-93337A, sc-93337B and sc-93337C.

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

POLR3E siRNA (h) is recommended for the inhibition of POLR3E 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

POLR3E (B-11): sc-365703 is recommended as a control antibody for monitoring of POLR3E 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 POLR3E gene expression knockdown using RT-PCR Primer: POLR3E (h)-PR: sc-93337-PR (20 µl). 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.