SANTA CRUZ BIOTECHNOLOGY, INC.

Pol II siRNA (m): sc-36291

BACKGROUND

RNA polymerase II (Pol II) is an enzyme that is composed of twelve subunits and is responsible for the transcription of protein-coding genes. Transcription initiation requires Pol II-mediated recruitment of transcription machinery to a target promoter, thereby allowing transcription to begin. The largest subunit of Pol II (referred to as RPB1 or RPB205) is a 1,840 amino acid protein that contains one C_2H_2-type zinc finger and a C-terminal domain comprised of several heptapeptide repeats. Although Pol II function requires the cooperation of all twelve subunits, the largest subunit conveys Pol II catalytic activity and, together with the second largest subunit, forms the active center of the Pol II enzyme. Additionally, the large subunit participates in forming the DNA-binding domain of Pol II, a groove that is necessary for transcription of the DNA template. Without proper function of the large subunit, mRNA synthesis and subsequent transcription elongation cannot occur.

REFERENCES

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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.
6. Cabart, P., et al. 2004. BRCA1 cooperates with NUFIP and P-TEFβ to activate transcription by RNA polymerase II. Oncogene 23: 5316-5329.
7. Svejstrup, J.Q. 2004. The RNA polymerase II transcription cycle: cycling through chromatin. Biochim. Biophys. Acta 1677: 64-73.
8. Cramer, P. 2004. Structure and function of RNA polymerase II. Adv. Protein Chem. 67: 1-42.

CHROMOSOMAL LOCATION

Genetic locus: Polr2a (mouse) mapping to 11 B3.

PRODUCT

Pol II 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 Pol II shRNA Plasmid (m): sc-36291-SH and Pol II shRNA (m) Lentiviral Particles: sc-36291-V as alternate gene silencing products.

For independent verification of Pol II (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-36291A, sc-36291B and sc-36291C.

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

Pol II siRNA (m) is recommended for the inhibition of Pol II 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

Pol II (CTD4H8): sc-47701 is recommended as a control antibody for monitoring of Pol II 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 Pol II gene expression knockdown using RT-PCR Primer: Pol II (m)-PR: sc-36291-PR (20 µl, 587 bp). 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.