SANTA CRUZ BIOTECHNOLOGY, INC.

POLR2B siRNA (h): sc-62962

BACKGROUND

RNA polymerase II (Pol II) is a multi-subunit complex responsible for catalyzing the transcription of DNA into RNA. POLR2B (polymerase (RNA) II (DNA directed) polypeptide B), also known as RPB2 (RNA polymerase II subunit B2), is a 1,174 amino acid protein that is the second largest subunit in the Pol II complex. POLR2B, along with the other Pol II subunits, uses the four ribonucleoside triphosphates as substrates to catalyze the transcription of DNA to RNA. Localized to the nucleus, POLR2B is one of at least three subunits that form a structure which is essential in maintaining contact between the active site of Pol II and the DNA template/newly synthesized RNA. In addition to POLR2B, this complex contains the central large cleft; a clamp element that opens and closes the cleft and functions to grab the incoming DNA template.

REFERENCES

1. Näär, A.M., et al. 2002. Human CRSP interacts with RNA polymerase II CTD and adopts a specific CTD-bound conformation. Genes Dev. 16: 1339-1344.
2. Langelier, M.F., et al. 2005. The highly conserved glutamic acid 791 of Rpb2 is involved in the binding of NTP and Mg(B) in the active center of human RNA polymerase II. Nucleic Acids Res. 33: 2629-2639.
3. Frøslev, T.G., et al. 2005. Lower level relationships in the mushroom genus *Cortinarius (Basidiomycota, Agaricales):* a comparison of RPB1, RPB2, and ITS phylogenies. Mol. Phylogenet. Evol. 37: 602-618.
4. Djupedal, I., et al. 2005. RNA Pol II subunit Rpb7 promotes centromeric transcription and RNAi-directed chromatin silencing. Genes Dev. 19: 2301-2306.
5. Thomas, M.M., et al. 2006. Molecular phylogeny of the palm genus *Chamaedorea*, based on the low-copy nuclear genes PRK and RPB2. Mol. Phylogenet. Evol. 38: 398-415.
6. Loo, A.H., et al. 2006. Low-copy nuclear DNA, phylogeny and the evolution of dichogamy in the betel nut palms and their relatives *(Arecinae; Arecaceae).* Mol. Phylogenet. Evol. 39: 598-618.
7. Malkus, A., et al. 2006. RNA polymerase II gene (RPB2) encoding the second largest protein subunit in *Phaeosphaeria nodorum* and *P. avenaria*. Mycol. Res. 110: 1152-1164.
8. Johnson, S.S., et al. 2007. Mammalian Maf1 is a negative regulator of transcription by all three nuclear RNA polymerases. Mol. Cell 26: 367-379.

CHROMOSOMAL LOCATION

Genetic locus: POLR2B (human) mapping to 4q12.

PRODUCT

POLR2B 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 POLR2B shRNA Plasmid (h): sc-62962-SH and POLR2B shRNA (h) Lentiviral Particles: sc-62962-V as alternate gene silencing products.

For independent verification of POLR2B (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-62962A, sc-62962B and sc-62962C.

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

POLR2B siRNA (h) is recommended for the inhibition of POLR2B 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

POLR2B (E-12): sc-166803 is recommended as a control antibody for monitoring of POLR2B 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 POLR2B gene expression knockdown using RT-PCR Primer: POLR2B (h)-PR: sc-62962-PR (20 µl). 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.