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# Pol I/II/III RPB6 shRNA (h) Lentiviral Particles: sc-45868-V

## BACKGROUND

RNA polymerase II (Pol II) is a multi-subunit enzyme responsible for the transcription of protein-coding genes. Transcription initiation requires recruitment of the complete transcription machinery to a promoter via solicitation by activators and chromatin remodeling factors. Pol II can coordinate 10 to 14 subunits. This complex interacts with the promoter regions of genes and a variety of elements and transcription factors. The DNA binding domain of the polymerase is a groove where TFIIB orients the DNA for unwinding and transcription.

## 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.
6. Cabart, P., et al. 2004. BRCA1 cooperates with NUFIP and P-TEFb 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.
9. Comai, L. 2004. Mechanism of RNA polymerase I transcription. *Adv. Protein Chem.* 67: 123-155.

## CHROMOSOMAL LOCATION

Genetic locus: POLR2F (human) mapping to 22q13.1.

## PRODUCT

Pol I/II/III RPB6 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Pol I/II/III RPB6 siRNA (h): sc-45868 and Pol I/II/III RPB6 shRNA Plasmid (h): sc-45868-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

Pol I/II/III RPB6 shRNA (h) Lentiviral Particles is recommended for the inhibition of Pol I/II/III RPB6 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

Pol I/II/III RPB6 (E-8): sc-271309 is recommended as a control antibody for monitoring of Pol I/II/III RPB6 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Pol I/II/III RPB6 gene expression knockdown using RT-PCR Primer: Pol I/II/III RPB6 (h)-PR: sc-45868-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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