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# Topo II $\beta$ shRNA (h2) Lentiviral Particles: sc-44161-V

## BACKGROUND

DNA topoisomerase I and II (Topo I and Topo II) are nuclear enzymes that regulate the topological structure of DNA in eukaryotic cells by transiently breaking and rejoining DNA strands. Eukaryotic topoisomerases are capable of relaxing both positive and negative supercoils, whereas prokaryotic topoisomerases relax only negative supercoils. DNA topoisomerases play a role in DNA replication, recombination and transcription, and have been identified as targets of numerous anticancer drugs. Topo I, a ubiquitously expressed, soluble enzyme, acts by introducing a transient break in one strand of DNA, while Topo II acts by making a transient double-strand break. Topo II is encoded by two different genes to generate two distinct isoforms that are designated Topo II $\alpha$  and Topo II $\beta$ . Topo II $\alpha$  and Topo II $\beta$  are largely homologous at their N-terminal three quarters, however, the C-terminal segments are considerably divergent, suggesting that these regions may mediate different cellular functions and account for the observed differential tissue expression patterns of the two isoforms.

## REFERENCES

1. D'Arpa, P., et al. 1988. cDNA cloning of human DNA topoisomerase I: catalytic activity of a 67.7 kDa carboxyl-terminal fragment. *Proc. Natl. Acad. Sci. USA* 85: 2543-2547.
2. Chung, T.D., et al. 1989. Characterization and immunological identification of cDNA clones encoding two human DNA topoisomerase II isozymes. *Proc. Natl. Acad. Sci. USA* 86: 9431-9435.
3. Austin, C.A., et al. 1990. Isolation and characterization of a human cDNA clone encoding a novel DNA topoisomerase II homologue from HeLa cells. *FEBS Letts.* 266: 115-117.
4. Kunze, N., et al. 1991. Structure of the human type I DNA topoisomerase gene. *J. Biol. Chem.* 266: 9610-9616.
5. Tan, K.B., et al. 1992. Topoisomerase II  $\alpha$  and topoisomerase II  $\beta$  genes: characterization and mapping to human chromosomes 17 and 3, respectively. *Cancer Res.* 52: 231-234.

## CHROMOSOMAL LOCATION

Genetic locus: TOP2B (human) mapping to 3p24.2.

## PRODUCT

Topo II $\beta$  shRNA (h2) 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 Topo II $\beta$  siRNA (h2): sc-44161 and Topo II $\beta$  shRNA Plasmid (h2): sc-44161-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

Topo II $\beta$  shRNA (h2) Lentiviral Particles is recommended for the inhibition of Topo II $\beta$  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

Topo II $\beta$  (B-6): sc-365952 is recommended as a control antibody for monitoring of Topo II $\beta$  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<sup>™</sup> compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker<sup>™</sup> 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<sup>™</sup> Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Topo II $\beta$  gene expression knockdown using RT-PCR Primer: Topo II $\beta$  (h2)-PR: sc-44161-PR (20  $\mu$ l, 481 bp). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## 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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