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Topo II β siRNA (h2): sc-44161

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 α and Topo II β . Topo II α and Topo II β 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 Lett.* 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 α and topoisomerase II β 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 β siRNA (h2) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Topo II β shRNA Plasmid (h2): sc-44161-SH and Topo II β shRNA (h2) Lentiviral Particles: sc-44161-V as alternate gene silencing products.

For independent verification of Topo II β (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44161A, sc-44161B and sc-44161C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Topo II β siRNA (h2) is recommended for the inhibition of Topo II β 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 μ M in 66 μ l. 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

Topo II β (A-12): sc-365071 is recommended as a control antibody for monitoring of Topo II β 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Topo II β gene expression knockdown using RT-PCR Primer: Topo II β (h2)-PR: sc-44161-PR (20 μ l, 481 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.