SANTA CRUZ BIOTECHNOLOGY, INC.

TOPORS siRNA (h): sc-92519

BACKGROUND

TOPORS (topoisomerase I binding, arginine/serine-rich), also known as LUN, RP31, P53BP3 or TP53BPL, is a 1,045 amino acid protein that contains one RING-type zinc finger and localizes to discrete nuclear foci. Expressed in a variety of tissues with highest expression in testis, TOPORS functions as a ubiquitin E3 ligase that, via its RING domain, can ubiquitinate proteins such as p53, thereby targeting them for proteasomal degradation. TOPORS expression can be induced by genotoxic agents (compounds that damage DNA), such as camptothecin and cisplatin, suggesting an important role for TOPORS in DNA damage repair pathways. In addition, TOPORS levels are decreased in lung and colon cancers, implicating TOPORS as a possible tumor suppressor. Defects in the gene encoding TOPORS are the cause of retinitis pigmentosa type 31 (RP31), a degenerative disease characterized by a loss of peripheral and, eventually, central vision.

REFERENCES

1. Zhou, R., et al. 1999. Identification of a novel gene encoding a p53-associated protein. Gene 235: 93-9101.
2. Haluska, P., et al. 1999. Interaction between human topoisomerase I and a novel RING finger/arginine-serine protein. Nucleic Acids Res. 27: 2538-2544.
3. Chu, D., et al. 2001. Cloning and characterization of LUN, a novel RING finger protein that is highly expressed in lung and specifically binds to a palindromic sequence. J. Biol. Chem. 276: 14004-14013.
4. Rasheed, Z.A., et al. 2002. The topoisomerase I-binding RING protein, topors, is associated with promyelocytic leukemia nuclear bodies. Exp. Cell Res. 277: 152-160.
5. Weger, S., et al. 2003. The DNA topoisomerase I binding protein TOPORS as a novel cellular target for SUMO-1 modification: characterization of domains necessary for subcellular localization and sumolation. Exp. Cell Res. 290: 13-27.
6. Rajendra, R., et al. 2004. TOPORS functions as an E3 ubiquitin ligase with specific E2 enzymes and ubiquitinates p53. J. Biol. Chem. 279: 36440-36444.

CHROMOSOMAL LOCATION

Genetic locus: TOPORS (human) mapping to 9p21.1.

PRODUCT

TOPORS 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 TOPORS shRNA Plasmid (h): sc-92519-SH and TOPORS shRNA (h) Lentiviral Particles: sc-92519-V as alternate gene silencing products.

For independent verification of TOPORS (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-92519A, sc-92519B and sc-92519C.

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

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

TOPORS (Y-30): sc-101182 is recommended as a control antibody for monitoring of TOPORS 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 TOPORS gene expression knockdown using RT-PCR Primer: TOPORS (h)-PR: sc-92519-PR (20 µl, 600 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.