

TopBP1 siRNA (m): sc-44393

BACKGROUND

Human DNA topoisomerase II binding protein 1 (TopBP1) contains eight BRCT motifs that are found in proteins regulating the DNA damage response, transcription, and replication. In addition, TopBP1 shares sequence similarity with the fission yeast Rad4/Cut5 protein and the budding yeast DPB11 protein, both of which are required for DNA damage control and/or replication checkpoint control. Phosphorylation of TopBP1 occurs in response to DNA double-strand breaks and replication blocks. TopBP1 forms nuclear foci and localizes to the sites of DNA damage or the arrested replication forks. Downregulation of TopBP1 leads to reduced cell survival, probably due to increased apoptosis. TopBP1 functions as a transcriptional coactivator by enhancing the human papillomavirus (HPV) transcription/replication factor E2. In addition, the HECT-domain ubiquitin ligase, hHYD, cooperates with TopBP1 in DNA damage response. TopBP1 specifically interacts with the C-terminal region of topoisomerase II β , which suggests a supportive role for TopBP1 in the catalytic reactions of topoisomerase II beta through transient breakages of DNA strands. The gene encoding TopBP1 maps to chromosome 3q22.1.

REFERENCES

1. Makiniemi, M., et al. 2001. BRCT domain-containing protein TopBP1 functions in DNA replication and damage response. *J. Biol. Chem.* 276: 30399-30406.
2. Honda, Y., et al. 2002. Cooperation of HECT-domain ubiquitin ligase hHYD and DNA topoisomerase II-binding protein for DNA damage response. *J. Biol. Chem.* 277: 3599-3605.
3. Boner, W., et al. 2002. A functional interaction between the human papillomavirus 16 transcription/replication factor E2 and the DNA damage response protein TopBP1. *J. Biol. Chem.* 277: 22297-22303.
4. Yamane, K., et al. 2002. A DNA damage-regulated BRCT-containing protein, TopBP1, is required for cell survival. *Mol. Cell. Biol.* 22: 555-566.
5. LocusLink Report (LocusID: 11073). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: Topbp1 (mouse) mapping to 9 F1.

PRODUCT

TopBP1 siRNA (m) 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 TopBP1 shRNA Plasmid (m): sc-44393-SH and TopBP1 shRNA (m) Lentiviral Particles: sc-44393-V as alternate gene silencing products.

For independent verification of TopBP1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44393A, sc-44393B and sc-44393C.

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

TopBP1 siRNA (m) is recommended for the inhibition of TopBP1 expression in mouse 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TopBP1 gene expression knockdown using RT-PCR Primer: TopBP1 (m)-PR: sc-44393-PR (20 μ l). 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.