

TopBP1 siRNA (h): sc-41068

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 β 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.

CHROMOSOMAL LOCATION

Genetic locus: TOPBP1 (human) mapping to 3q22.1.

PRODUCT

TopBP1 siRNA (h) is a target-specific 19-25 nt siRNA 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 (h): sc-41068-SH and TopBP1 shRNA (h) Lentiviral Particles: sc-41068-V as alternate gene silencing 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 (h) is recommended for the inhibition of TopBP1 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

TopBP1 (B-7): sc-271043 is recommended as a control antibody for monitoring of TopBP1 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 TopBP1 gene expression knockdown using RT-PCR Primer: TopBP1 (h)-PR: sc-41068-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Hahm, S.H., et al. 2012. Human MutY homolog induces apoptosis in etoposide-treated HEK293 cells. *Oncol. Lett.* 4: 1203-1208.
2. Lv, Y., et al. 2016. TopBP1 contributes to the chemoresistance in non-small cell lung cancer through upregulation of p53. *Drug Des. Devel. Ther.* 10: 3053-3064.
3. Karanika, S., et al. 2017. Targeting DNA damage response in prostate cancer by inhibiting androgen receptor-Cdc6-ATR-Chk1 signaling. *Cell Rep.* 18: 1970-1981.
4. Lv, Y., et al. 2017. Calcein-acetoxymethyl ester enhances the antitumor effects of doxorubicin in nonsmall cell lung cancer by regulating the TopBP1/p53RR pathway. *Anticancer Drugs* 28: 861-868.

RESEARCH USE

For research use only, not for use in diagnostic procedures.