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Rad51B siRNA (h): sc-45855

BACKGROUND

Rad52 family members (Rad50, Rad51B/C/D, Rad52, Rad54, MRE11) mediate DNA double-strand break repair (DSBR) for DNA damage that otherwise could cause cell death, mutation or neoplastic transformation. Rad51 (RECA, BRCC5) interacts with BRCA1 and BRCA2 to influence subcellular localization and cellular response to DNA damage. BRCA2 inactivation may be a key event leading to genomic instability and tumorigenesis from deregulation of Rad51. Rad52 forms a heptameric ring that binds single-stranded DNA ends and catalyzes DNA-DNA interaction necessary for the annealing of complementary strands. Rad52 can interact with Rad51. Rad54A of the DEAD-like helicase superfamily binds to double-strand DNA and induces a DNA topological change, which is thought to facilitate homologous DNA pairing and stimulate DNA recombination. Rad54B of the DEAD-like helicase superfamily binds to double-stranded DNA and displays ATPase activity in the presence of DNA. Rad54B is abundant in testis and spleen, and mutations of this gene occur in primary lymphoma and colon cancer. MRE11 (meiotic recombination 11, ATLD, HNGS1) is a nuclear 3'-5' exonuclease/endonuclease that associates with Rad50 and influences homologous recombination, telomere length maintenance, and DNA double-strand break repair. MRE11 is most abundant in proliferating tissues.

REFERENCES

1. Connell, P.P., et al. 2004. A hot spot for Rad51C interactions revealed by a peptide that sensitizes cells to cisplatin. *Cancer Res.* 64: 3002-3005.
2. Forget, A.L., et al. 2004. Xrcc3 is recruited to DNA double strand breaks early and independent of Rad51. *J. Cell Biochem.* 93: 429-436.
3. Lio, Y.C., et al. 2004. Human Rad51C deficiency destabilizes XRCC3, impairs recombination, and radiosensitizes S/G₂-phase cells. *J. Biol. Chem.* 279: 42313-42320.
4. Sasaki, M.S., et al. 2004. Recombination repair pathway in the maintenance of chromosomal integrity against DNA interstrand crosslinks. *Cytogenet. Genome. Res.* 104: 28-34.
5. Yokoyama, H., et al. 2004. Preferential binding to branched DNA strands and strand-annealing activity of the human Rad51B, Rad51C, Rad51D and XRCC2 protein complex. *Nucleic Acids Res.* 32: 2556-2565.

CHROMOSOMAL LOCATION

Genetic locus: RAD51B (human) mapping to 14q24.1.

PRODUCT

Rad51B 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Rad51B shRNA Plasmid (h): sc-45855-SH and Rad51B shRNA (h) Lentiviral Particles: sc-45855-V as alternate gene silencing products.

For independent verification of Rad51B (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-45855A, sc-45855B and sc-45855C.

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

Rad51B siRNA (h) is recommended for the inhibition of Rad51B 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

Rad51B (F-12): sc-377192 is recommended as a control antibody for monitoring of Rad51B 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™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ 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™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Rad51B gene expression knockdown using RT-PCR Primer: Rad51B (h)-PR: sc-45855-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.