

Rad50 siRNA (h): sc-37397

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. Tsukamoto, Y., et al. 1996. Effects of mutations of Rad50, Rad51, Rad52, and related genes on illegitimate recombination in *Saccharomyces cerevisiae*. *Genetics* 142: 383-391.
2. Zhong, Q., et al. 2002. Deficient nonhomologous end-joining activity in cell-free extracts from BRCA1-null fibroblasts. *Cancer Res.* 62: 3966-3970.

CHROMOSOMAL LOCATION

Genetic locus: RAD50 (human) mapping to 5q31.1.

PRODUCT

Rad50 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 Rad50 shRNA Plasmid (h): sc-37397-SH and Rad50 shRNA (h) Lentiviral Particles: sc-37397-V as alternate gene silencing products.

For independent verification of Rad50 (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-37397A, sc-37397B and sc-37397C.

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

Rad50 siRNA (h) is recommended for the inhibition of Rad50 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

Rad50 (G-2): sc-74460 is recommended as a control antibody for monitoring of Rad50 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 Rad50 gene expression knockdown using RT-PCR Primer: Rad50 (h)-PR: sc-37397-PR (20 μ l, 471 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Shin, M.H., et al. 2012. ATM-dependent phosphorylation of the checkpoint clamp regulates repair pathways and maintains genomic stability. *Cell Cycle* 11: 1796-1803.

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.