

Nibrin siRNA (h): sc-36061

BACKGROUND

DNA repair proteins are necessary for the maintenance of chromosome integrity and are involved in the elimination of premutagenic lesions from DNA. The DNA repair proteins Rad51 and Rad52 are key components of the double-strand-break repair (DSBR) pathway. Rad51 is essential for mitotic and meiotic recombination and its mutation in yeast and mammalian cells results in chromosome loss. Overexpression of Rad52 confers resistance to ionizing radiation and induces homologous intrachromosomal recombination. Rad52 is thought to be involved in an early stage of Rad51-mediated recombination. Additional proteins involved in the pathway include Dmc1 and Nibrin. Dmc1 is specifically involved in meiotic recombination. Nibrin, which complexes with MRE11 and Rad50, is absent in Nijmegen breakage syndrome (NBS) patients.

CHROMOSOMAL LOCATION

Genetic locus: NBN (human) mapping to 8q21.3.

PRODUCT

Nibrin 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 Nibrin shRNA Plasmid (h): sc-36061-SH and Nibrin shRNA (h) Lentiviral Particles: sc-36061-V as alternate gene silencing products.

For independent verification of Nibrin (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-36061A, sc-36061B and sc-36061C.

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

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

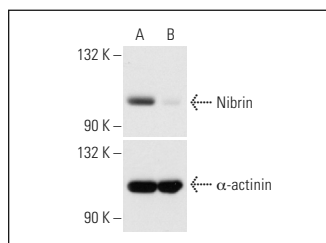
Nibrin (B-5): sc-515069 is recommended as a control antibody for monitoring of Nibrin gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

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 Nibrin gene expression knockdown using RT-PCR Primer: Nibrin (h)-PR: sc-36061-PR (20 μ l, 518 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

DATA



Nibrin siRNA (h): sc-36061. Western blot analysis of Nibrin expression in non-transfected control (A) and Nibrin siRNA transfected (B) HeLa cells. Blot probed with Nibrin (C-19): sc-8580. α -actinin (H-2): sc-17829 used as specificity and loading control.

SELECT PRODUCT CITATIONS

- 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.
- Velichko, A.K., et al. 2019. Hypoosmotic stress induces R loop formation in nucleoli and ATR/ATM-dependent silencing of nucleolar transcription. *Nucleic Acids Res.* 47: 6811-6825.

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.