SANTA CRUZ BIOTECHNOLOGY, INC.

NSE1 siRNA (h): sc-93235

BACKGROUND

Breaks in double stranded DNA often arise during DNA replication or as a result of exposure to DNA-damaging agents. Quick and accurate repair of these breaks is crucial for cell survival and genomic stability. Structural maintenance of chromosomes (SMC) family members form heterodimeric complexes that modulate sister chromatid cohesion and chromosome condensation during mitosis. SMC5 and SMC6 play a crucial role in DNA repair as they form a complex with six conserved nonSMC subunits, including a ubiquitin E3 ligase NSE1 and a SUMO ligase NSE2. Specifically, this complex is crucial for sister chromatid homologous recombination DNA repair and also for prevention of chromosomal rearrangements. The NSE1 protein contains a RING-like motif that promotes DNA repair functions of the SMC5/SMC6 complex and full deletion of NSE1 is lethal to cells. NSE2 stimulates sumoylation of SMC6 and the DNA repair protein TRAX. Depletion of the NSE2 protein by RNA interference leaves the cell vulnerable to DNA damage-induced apoptosis.

REFERENCES

1. Lehmann, A.R. 2005. The role of SMC proteins in the responses to DNA damage. DNA Repair 4: 309-314.
2. Lee, K.M. and O'Connell, M.J. 2005. A new SUMO ligase in the DNA damage response. DNA Repair 5: 138-141.
3. Potts, P.R. and Yu, H. 2005. Human MMS21/NSE2 is a SUMO ligase required for DNA repair. Mol. Cell. Biol. 25: 7021-7032.
4. Watanabe, Y. 2005. The importance of being SMC5/6. Nat. Cell Biol. 7: 329-331.
5. Eydmann, T., et al. 2005. SMC5 and SMC6 genes are required for the segregation of repetitive chromosome regions. Nat. Cell Biol. 7: 412-419.
6. De Piccoli, G., et al. 2006. SMC5-SMC6 mediate DNA double-strand-break repair by promoting sister-chromatid recombination. Nat. Cell Biol. 8: 1032-1034.
7. Lindroos, H.B., et al. 2006. Chromosomal association of the SMC5/6 complex reveals that it functions in differently regulated pathways. Mol. Cell 22: 755-767.

CHROMOSOMAL LOCATION

Genetic locus: NSMCE1 (human) mapping to 16p12.1.

PRODUCT

NSE1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NSE1 shRNA Plasmid (h): sc-93235-SH and NSE1 shRNA (h) Lentiviral Particles: sc-93235-V as alternate gene silencing products.

For independent verification of NSE1 (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-93235A, sc-93235B and sc-93235C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NSE1 siRNA (h) is recommended for the inhibition of NSE1 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 µM in 66 µl. 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

NSE1 (D-2): sc-376585 is recommended as a control antibody for monitoring of NSE1 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 NSE1 gene expression knockdown using RT-PCR Primer: NSE1 (h)-PR: sc-93235-PR (20 µl). 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.