

NHP2 siRNA (h): sc-38300

BACKGROUND

Dyskerin (NAP57) associates with the chaperone protein Nopp140 and forms a small ribonucleoprotein particle with GAR1 (NOLA1), NHP2 (NOLA2) and Nop10 for the isomerization of uridine to pseudouridine. GAR1, NHP2 and dyskerin localize to the dense fibrillar component of the nucleolus and in nuclear Cajal bodies. The dyskerin gene maps to chromosome Xq28. Missense mutations in the dyskerin gene interfere with normal nuclear localization of dyskerin and cause Dyskeratosis congenita (DKC). DKC is a rare, X-linked bone marrow disorder characterized by cutaneous hyperpigmentation, dystrophy of the nails, atrophy of the testicles and leukoplakia of the oral mucosa. The GAR1 gene maps to chromosome 4q25. The NHP2 gene maps to chromosome 5q35.3 and encodes a 155-amino acid protein.

REFERENCES

1. Hassock, S., Vetrie, D. and Giannelli, F. 1999. Mapping and characterization of the X-linked dyskeratosis congenita (DKC) gene. *Genomics* 55: 21-27.
2. Heiss, N.S., Girod, A., Salowsky, R., Wiemann, S., Pepperkok, R. and Poustka, A. 1999. Dyskerin localizes to the nucleolus and its mislocalization is unlikely to play a role in the pathogenesis of dyskeratosis congenita. *Hum. Mol. Genet.* 8: 2515-2524.
3. Dragon, F., Pogacic, V. and Filipowicz, W. 2000. *In vitro* assembly of human H/ACA small nucleolar RNPs reveals unique features of U17 and telomerase RNAs. *Mol. Cell. Biol.* 20: 3037-3048.
4. Pogacic, V., Dragon, F. and Filipowicz, W. 2000. Human H/ACA small nucleolar RNPs and telomerase share evolutionarily conserved proteins NHP2 and NOP10. *Mol. Cell. Biol.* 20: 9028-9040.
5. Wang, C., et al. 2002. Immunopurified small nucleolar ribonucleoprotein particles pseudouridylate rRNA independently of their association with phosphorylated Nopp140. *Mol. Cell. Biol.* 22: 8457-8466.
6. LocusLink Report (LocusID: 54433). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: NHP2 (human) mapping to 5q35.3.

PRODUCT

NHP2 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 NHP2 shRNA Plasmid (h): sc-38300-SH and NHP2 shRNA (h) Lentiviral Particles: sc-38300-V as alternate gene silencing products.

For independent verification of NHP2 (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-38300A, sc-38300B and sc-38300C.

PROTOCOLS

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

NHP2 siRNA (h) is recommended for the inhibition of NHP2 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

NHP2 (H-9): sc-398430 is recommended as a control antibody for monitoring of NHP2 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 NHP2 gene expression knockdown using RT-PCR Primer: NHP2 (h)-PR: sc-38300-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.