

Rtn-3 siRNA (h): sc-42219

BACKGROUND

The Reticulon (Rtn) family consists of four members: Rtn-1 (also designated neuroendocrine-specific protein or NSP), Rtn-2 (also designated NSP-like-1), Rtn-3 (also designated NSP-like-2) and Nogo (also designated Rtn-4A). Reticulon proteins are anchored to the membranes of the endoplasmic reticulum through their common C-terminal regions. Localized on human chromosome 14q23.1, the gene encoding Rtn-1 is expressed as three isoforms: Rtn-1A (NSP-A), Rtn-1B (NSP-B) and Rtn-1C (NSP-C). The gene encoding human Rtn-2 is located on chromosome 19q13 and also encodes three isoforms. Rtn-2-A and Rtn-2-C are produced by the use of alternative initiation sites, whereas Rtn-2-B is an alternative splice variant of the Rtn-2-A isoform. Rtn-2-A and Rtn-2-B are highly expressed in brain, while Rtn-2-C is primarily expressed in skeletal muscle. The human Rtn-3 gene is located on chromosome 11q13.1 and is widely expressed, with the highest expression being in brain.

REFERENCES

1. Senden, N.H., et al. 1994. Subcellular localization and supramolecular organization of neuroendocrine-specific protein B (NSP-B) in small cell lung cancer. *Eur. J. Cell Biol.* 65: 341-353.
2. van de Velde, H. J., et al. 1994. NSP-encoded reticulons are neuroendocrine markers of a novel category in human lung cancer diagnosis. *Cancer Res.* 54: 4769-4776.
3. Geisler, J.G., et al. 1998. Molecular cloning of a novel mouse gene with predominant muscle and neural expression. *Mamm. Genome* 9: 274-282.
4. Roebroek, A.J., et al. 1998. cDNA cloning, genomic organization, and expression of the human RTN2 gene, a member of a gene family encoding reticulons. *Genomics* 51: 98-106.
5. Hens, J., et al. 1998. Neuronal differentiation is accompanied by NSP-C expression. *Cell Tissue Res.* 292: 229-237.
6. Moreira, E.F., et al. 1999. Cloning of a novel member of the reticulon gene family (RTN3): gene structure and chromosomal localization to 11q13. *Genomics* 58: 73-81.
7. GrandPre, T., et al. 2000. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. *Nature* 403: 439-444.

CHROMOSOMAL LOCATION

Genetic locus: RTN3 (human) mapping to 11q13.1.

PRODUCT

Rtn-3 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 Rtn-3 shRNA Plasmid (h): sc-42219-SH and Rtn-3 shRNA (h) Lentiviral Particles: sc-42219-V as alternate gene silencing products.

For independent verification of Rtn-3 (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-42219A, sc-42219B and sc-42219C.

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

Rtn-3 siRNA (h) is recommended for the inhibition of Rtn-3 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

Rtn-3 (F-6): sc-374599 is recommended as a control antibody for monitoring of Rtn-3 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 MarkerTM 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 Rtn-3 gene expression knockdown using RT-PCR Primer: Rtn-3 (h)-PR: sc-42219-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.

PROTOCOLS

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