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Gemin5 siRNA (h): sc-42131

BACKGROUND

Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease characterized by loss of motor neurons in the spinal cord. SMA is caused by deletion or loss-of-function mutations in the SMN (survival of motor neuron) gene. Gemin5, the protein product of human chromosome 5q33.3, associates directly with SMN and is a part of the SMN complex containing Gemin2, Gemin3, Gemin4 and Gemin6 as well as several spliceosomal snRNP proteins. The SMN complex plays an essential role in spliceosomal snRNP assembly in the cytoplasm and is required for pre-mRNA splicing of the nucleus. The SMN complex is found in both the cytoplasm and the nucleus. The nuclear form is concentrated in subnuclear bodies called gems (Gemini of the coiled bodies). Gemin5 interacts with several snRNP core proteins including SmB, SmD1, SmD2, SmD3 and SmE. The amino terminal half of Gemin5 contains 13 WD repeat domains and a coiled-coil motif near the C-terminus.

REFERENCES

1. Fischer, U., et al. 1997. The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. *Cell* 90: 1023-1029.
2. Coovert, D., et al. 1997. The survival motor neuron protein in spinal muscular atrophy. *Hum. Mol. Genet* 6: 1205-1214.
3. Monani, U., et al. 1999. A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. *Hum. Mol. Genet.* 8: 1177-1183.
4. Meister, G., et al. 2000. Characterization of a nuclear 20S complex containing the survival of motor neurons (SMN) protein and a specific subset of spliceosomal Sm proteins. *Hum. Mol. Genet.* 9: 1977-1986.
5. Mourelatos, Z., et al. 2001. SMN interacts with a novel family of hnRNP and spliceosomal proteins. *EMBO J.* 20: 5443-5452.
6. Gubitza, A., et al. 2002. Gemin5, a novel WD repeat protein component of the SMN complex that binds Sm Proteins. *J. Biol. Chem.* 277: 5631-5636.
7. LocusLink Report (LocusID: 25929). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: GEMIN5 (human) mapping to 5q33.2.

PRODUCT

Gemin5 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 Gemin5 shRNA Plasmid (h): sc-42131-SH and Gemin5 shRNA (h) Lentiviral Particles: sc-42131-V as alternate gene silencing products.

For independent verification of Gemin5 (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-42131A, sc-42131B and sc-42131C.

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

Gemin5 siRNA (h) is recommended for the inhibition of Gemin5 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

Gemin5 (10G11): sc-136200 is recommended as a control antibody for monitoring of Gemin5 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 Gemin5 gene expression knockdown using RT-PCR Primer: Gemin5 (h)-PR: sc-42131-PR (20 μ l, 573 bp). 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.