

Otospiralin siRNA (h): sc-62725

BACKGROUND

Otospiralin is an 89 amino acid inner ear-specific protein encoded by the OTOS gene. Otospiralin is synthesized by fibrocytes of spiral limbus and spiral ligament in the cochlea. Fibrocytes are responsible for maintaining inner ear homeostasis and impairment or alteration of these cells may lead to deterioration of auditory function. Degeneration of fibrocytes due to the absence of Otospiralin leads to irreversible deafness in guinea pigs and moderate deafness in mice. Loss of function in hair cells of the inner ear may also be caused by the downregulation of Otospiralin. Otospiralin is conserved from fish to mammals. It shares homology with gag p30 core shell and SARS of type C retroviruses. One isoform is produced due to alternative splicing.

REFERENCES

1. Gratton, M.A., et al. 1996. Characterization and development of an inner ear type I fibrocyte cell culture. *Hear. Res.* 99: 71-78.
2. Delprat, B., et al. 2002. Downregulation of otospiralin, a novel inner ear protein, causes hair cell degeneration and deafness. *J. Neurosci.* 22: 1718-1725.
3. Lavigne-Rebillard, M., et al. 2003. Gene structure, chromosomal localization, and mutation screening of the human gene for the inner ear protein otospiralin. *Neurogenetics* 4: 137-140.
4. Online Mendelian Inheritance in Man, OMIM™. 2003. Johns Hopkins University, Baltimore, MD. MIM Number: 607877. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
5. Pompeia, C., et al. 2004. Gene expression profile of the mouse organ of Corti at the onset of hearing. *Genomics* 83: 1000-1011.
6. Caravelli, A., et al. 2004. Down-regulation of otospiralin mRNA in response to acoustic stress in guinea porcine. *Hear. Res.* 198: 36-40.
7. Delprat, B., et al. 2005. Deafness and cochlear fibrocyte alterations in mice deficient for the inner ear protein otospiralin. *Mol. Cell. Biol.* 25: 847-853.

CHROMOSOMAL LOCATION

Genetic locus: OTOS (human) mapping to 2q37.3.

PRODUCT

Otospiralin 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 Otospiralin shRNA Plasmid (h): sc-62725-SH and Otospiralin shRNA (h) Lentiviral Particles: sc-62725-V as alternate gene silencing products.

For independent verification of Otospiralin (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-62725A, sc-62725B and sc-62725C.

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

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

Otospiralin (A-12): sc-393239 is recommended as a control antibody for monitoring of Otospiralin 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 Otospiralin gene expression knockdown using RT-PCR Primer: Otospiralin (h)-PR: sc-62725-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.