SANTA CRUZ BIOTECHNOLOGY, INC.

Whirlin siRNA (h): sc-61800

BACKGROUND

Whirlin is a cytoplasmic PDZ domain-containing protein that plays a role in elongation and maintenance of stereocilia, mechanosensory organelles located in hair cells of the inner ear. Whirlin co-localizes with Actin filaments and is primarily detected in cochlear hair cells. It is connected to the dynamic Usher protein interactome and has a pleiotropic function in both the retina and the inner ear. Myosin XVa is a motor protein that accociates with the second and third PDZ domain of Whirlin through its C-terminal PDZ-ligand. Myosin XVa then delivers Whirlin to the tips of stereocilia, which are subsequently elongated. p55 also interacts with Whirlin, and mutations in DFNB31, the Whirlin gene, lead to an early ablation of p55 labeling of stereocilia, which may cause recessive hearing loss in rats and humans.

REFERENCES

1. Belyantseva, I.A., et al. 2003. Stereocilia: the long and the short of it. Trends Mol. Med. 9: 458-461.
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3. Delprat, B., et al. 2005. Myosin XVa and Whirlin, two deafness gene products required for hair bundle growth, are located at the stereocilia tips and interact directly. Hum. Mol. Genet. 14: 401-410.
4. Adato, A., et al. 2005. Usherin, the defective protein in Usher syndrome type IIA, is likely to be a component of interstereocilia ankle links in the inner ear sensory cells. Hum. Mol. Genet. 14: 3921-3932.
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6. Rzadzinska, A., et al. 2005. Balanced levels of Espin are critical for stereociliary growth and length maintenance. Cell Motil. Cytoskeleton 62: 157-165.
7. Tlili, A., et al. 2005. Identification of a novel frameshift mutation in the DFNB31/WHRN gene in a Tunisian consanguineous family with hereditary non-syndromic recessive hearing loss. Hum. Mutat. 25: 503.
8. Mburu, P., et al. 2006. Whirlin complexes with p55 at the stereocilia tip during hair cell development. Proc. Natl. Acad. Sci. USA 103: 10973-10978.

CHROMOSOMAL LOCATION

Genetic locus: DFNB31 (human) mapping to 9q32.

PRODUCT

Whirlin 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 Whirlin shRNA Plasmid (h): sc-61800-SH and Whirlin shRNA (h) Lentiviral Particles: sc-61800-V as alternate gene silencing products.

For independent verification of Whirlin (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-61800A, sc-61800B and sc-61800C.

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

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

Whirlin (G-8): sc-365250 is recommended as a control antibody for monitoring of Whirlin 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 Whirlin gene expression knockdown using RT-PCR Primer: Whirlin (h)-PR: sc-61800-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.