SANTA CRUZ BIOTECHNOLOGY, INC.

Otoferlin siRNA (h): sc-61269

BACKGROUND

Otoferlin is a single-pass type II membrane protein composed of 1,230 amino acid residues. Otoferlin exists in four isoforms; isoform 1 is the full-length Otoferlin protein, whereas isoforms 2-4 are shorter versions of the protein. Expression of isoforms 1 and 3 is demonstrated in adult brain, while isoform 2 is expressed in the fetus, adult brain, heart, placenta, skeletal muscle and kidney tissues. Otoferlin has three C2 domains and a single carboxy-terminal transmembrane domain. The Otoferlin gene, OTOF, and its surrounding genes map to 2p23.2, and the 5' region of OTOF is centromeric. Mutations in the OTOF gene are implicated in deafness. Otoferlin is homologous to the *C. elegans* spermatogenesis factor FER-1 and to human dysferlin, implicating the involvement of Otoferlin in the Ca^{2+}-triggered synaptic vesicle-plasma membrane fusion.

REFERENCES

1. Starr, A., et al. 1998. Transient deafness due to temperature-sensitive auditory neuropathy. Ear Hear. 19: 169-179.
2. Yasunaga, S., et al. 1999. A mutation in OTOF, encoding otoferlin, a FER-1-like protein, causes DFNB9, a nonsyndromic form of deafness. Nat. Genet. 21: 363-369.
3. Adato, A., et al. 2000. Deafness heterogeneity in a Druze isolate from the Middle East: novel OTOF and PDS mutations, low prevalence of GJB2 35delG mutation and indication for a new DFNB locus. Eur. J. Hum. Genet. 8: 437-442.
4. Migliosi, V., et al. 2002. Q829X, a novel mutation in the gene encoding otoferlin (OTOF), is frequently found in Spanish patients with prelingual non-syndromic hearing loss. J. Med. Genet. 39: 502-506.
5. Mirghomizadeh, F., et al. 2002. Substitutions in the conserved C2C domain of otoferlin cause DFNB9, a form of nonsyndromic autosomal recessive deafness. Neurobiol. Dis. 10: 157-164.
6. Mirghomizadeh, F., et al. 2002. Uncommon cytidine-homopolymer dimorphism in 5'-UTR of the human otoferlin gene. Int. J. Mol. Med. 11: 63-64.
7. Rodríguez-Ballesteros, M., et al. 2003. Auditory neuropathy in patients carrying mutations in the otoferlin gene (OTOF). Hum. Mutat. 22: 451-456.

CHROMOSOMAL LOCATION

Genetic locus: OTOF (human) mapping to 2p23.3.

PRODUCT

Otoferlin 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 Otoferlin shRNA Plasmid (h): sc-61269-SH and Otoferlin shRNA (h) Lentiviral Particles: sc-61269-V as alternate gene silencing products.

For independent verification of Otoferlin (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-61269A, sc-61269B and sc-61269C.

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

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

Otoferlin (C-12): sc-271092 is recommended as a control antibody for monitoring of Otoferlin 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 Otoferlin gene expression knockdown using RT-PCR Primer: Otoferlin (h)-PR: sc-61269-PR (20 µl, 543 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.

PROTOCOLS

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