

Usherin siRNA (h): sc-61762

BACKGROUND

Usherin is a membrane glycoprotein mainly expressed in all capillary and structural basement membranes of the retina and inner ear. It is also found in the spleen, oviduct, epididymis, testis and intestines. Usherin is crucial for inner ear and retina development and also functions to maintain homeostasis in many other organs. Fibronectin interacts with the LE domain of Usherin in retinal basement membranes and Collagen IV stabilizes Usherin in testicular basement membranes. Mutations in Usherin have been associated with Usher syndrome type IIa, characterized by moderate to severe congenital sensorineural hearing loss with retinitis pigmentosa. These symptoms are caused by the disruption of hair bundle links—mediated adhesion forces that are crucial for the correct organization of growing hair bundles.

REFERENCES

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4. Pennings, R.J., et al. 2004. USH2A mutation analysis in 70 Dutch families with Usher syndrome type II. *Hum. Mutat.* 24: 185.
5. Adato, A., et al. 2005. Usherin, the defective protein in a component of interstereocilia ankle links in the inner ear sensory cells. *Hum. Mol. Genet.* 14: 3921-3932.
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7. Bhattacharya, G. and Cosgrove, D. 2005. Evidence for functional importance of Usherin/Fibronectin interactions in retinal basement membranes. *Biochemistry* 44: 11518-11524.
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CHROMOSOMAL LOCATION

Genetic locus: USH2A (human) mapping to 1q41.

PRODUCT

Usherin 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 Usherin shRNA Plasmid (h): sc-61762-SH and Usherin shRNA (h) Lentiviral Particles: sc-61762-V as alternate gene silencing products.

For independent verification of Usherin (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-61762A, sc-61762B and sc-61762C.

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

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

Usherin (G-6): sc-515555 is recommended as a control antibody for monitoring of Usherin 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 Usherin gene expression knockdown using RT-PCR Primer: Usherin (h)-PR: sc-61762-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.