SANTA CRUZ BIOTECHNOLOGY, INC.

PCDH15 siRNA (h): sc-90494

BACKGROUND

Protocadherins are a large family of cadherin-like cell adhesion proteins that are involved in the establishment and maintenance of neuronal connections in the brain. There are three protocadherin gene clusters, designated α, β and γ, all of which contain multiple tandemly arranged genes. PCDH15 (protocadherin 15), also known as USH1F or DFNB23, is a 1,955 amino acid single-pass type I membrane protein that contains 11 cadherin domains and exists as multiple alternatively spliced isoforms. Expressed in testis, brain, lung, kidney and spleen, PCDH15 functions as a calcium-dependent cell-adhesion protein that is crucial for the maintenance of normal cochlear and retinal function. Defects in the gene encoding PCDH15 are associated with Usher syndrome type 1F (USH1F), Usher syndrome type 1D/F (USH1DF) and non-syndromic sensorineural deafness autosomal recessive type 23 (DFNB23), all of which are associated with deafness. Multiple isoforms of PCDH15 exist due to alternative splicing events.

REFERENCES

1. Ahmed, Z.M., et al. 2001. Mutations of the protocadherin gene PCDH15 cause Usher syndrome type 1F. Am. J. Hum. Genet. 69: 25-34.
2. Alagramam, K.N., et al. 2001. Mutations in the novel protocadherin PCDH15 cause Usher syndrome type 1F. Hum. Mol. Genet. 10: 1709-1718.
3. Ahmed, Z.M., et al. 2003. PCDH15 is expressed in the neurosensory epithelium of the eye and ear and mutant alleles are responsible for both USH1F and DFNB23. Hum. Mol. Genet. 12: 3215-3223.
4. Zheng, Q.Y., et al. 2005. Digenic inheritance of deafness caused by mutations in genes encoding cadherin 23 and protocadherin 15 in mice and humans. Hum. Mol. Genet. 14: 103-111.
5. Zheng, Q.Y., et al. 2006. A new spontaneous mutation in the mouse protocadherin 15 gene. Hear. Res. 219: 110-120.
6. Alagramam, K.N., et al. 2007. Promoter, alternative splice forms, and genomic structure of protocadherin 15. Genomics 90: 482-492.
7. Le Guedard, S., et al. 2007. Large genomic rearrangements within the PCDH15 gene are a significant cause of USH1F syndrome. Mol. Vis. 13: 102-107.

CHROMOSOMAL LOCATION

Genetic locus: PCDH15 (human) mapping to 10q21.1.

PRODUCT

PCDH15 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 PCDH15 shRNA Plasmid (h): sc-90494-SH and PCDH15 shRNA (h) Lentiviral Particles: sc-90494-V as alternate gene silencing products.

For independent verification of PCDH15 (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-90494A, sc-90494B and sc-90494C.

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

PCDH15 siRNA (h) is recommended for the inhibition of PCDH15 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

PCDH15 (H-3): sc-377235 is recommended as a control antibody for monitoring of PCDH15 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 PCDH15 gene expression knockdown using RT-PCR Primer: PCDH15 (h)-PR: sc-90494-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.