

claudin-5 siRNA (h): sc-43044

BACKGROUND

The claudin superfamily consists of many structurally related proteins in humans. These proteins are important structural and functional components of tight junctions in paracellular transport. Claudins are located in both epithelial and endothelial cells in all tight junction-bearing tissues. Three classes of proteins are known to localize to tight junctions, including the claudins, Occludin and Junction adhesion molecule. Claudins, which consist of four transmembrane domains and two extracellular loops make up tight junction strands. Claudin expression is highly restricted to specific regions of different tissues and may have an important role in transcellular transport through tight junctions. Claudin-5 is expressed in the endothelial junctions of the rat liver and in junctions of acinar cells of the pancreas. Human claudin-5 is abundantly expressed in adult lung, heart and skeletal muscle and is deleted in patients with velocardiofacial syndrome, which is characterized by cleft palate, facial dysmorphism and conotruncal heart defects.

REFERENCES

1. Fanning, A.S., et al. 1999. Transmembrane proteins in the tight junction barrier. *J. Am. Soc. Nephrol.* 10: 1337-1345.
2. Fujita, K., et al. 2000. Clostridium perfringens enterotoxin binds to the second extracellular loop of claudin-3, a tight junction integral membrane protein. *FEBS Lett.* 476: 258-261.
3. Heiskala, M., et al. 2001. The roles of Claudin superfamily proteins in paracellular transport. *Traffic* 2: 93-98.
4. Nishiyama, R., et al. 2001. IL-2 receptor β subunit dependent and independent regulation of intestinal epithelial tight junctions. *J. Biol. Chem.* 276: 35571-35580.

CHROMOSOMAL LOCATION

Genetic locus: CLDN5 (human) mapping to 22q11.21.

PRODUCT

claudin-5 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 claudin-5 shRNA Plasmid (h): sc-43044-SH and claudin-5 shRNA (h) Lentiviral Particles: sc-43044-V as alternate gene silencing products.

For independent verification of claudin-5 (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-43044A, sc-43044B and sc-43044C.

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

claudin-5 siRNA (h) is recommended for the inhibition of claudin-5 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

claudin-5 (A-12): sc-374221 is recommended as a control antibody for monitoring of claudin-5 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 claudin-5 gene expression knockdown using RT-PCR Primer: claudin-5 (h)-PR: sc-43044-PR (20 μ l, 448 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

SELECT PRODUCT CITATIONS

1. Gross, C.M., et al. 2014. Sox18 preserves the pulmonary endothelial barrier under conditions of increased shear stress. *J. Cell. Physiol.* 229: 1802-1816.
2. Gross, C.M., et al. 2018. LPS-induced acute lung injury involves NF κ B-mediated downregulation of SOX18. *Am. J. Respir. Cell Mol. Biol.* 58: 614-624.

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.