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connexin 50 siRNA (h): sc-43083

BACKGROUND

The connexin family of proteins form hexameric complexes called "connexons" that facilitate movement of low molecular weight proteins between cells via gap junctions. Connexin proteins share a common topology of four transmembrane α -helical domains, two extracellular loops, a cytoplasmic loop and cytoplasmic N- and C-termini. Many of the key functional differences arise from specific amino-acid substitutions in the most highly conserved domains, the transmembrane and extracellular regions. Each of the approximately 20 connexin isoforms produces channels with distinct permeabilities and electrical and chemical sensitivities; therefore, one connexin usually cannot fully substitute for another. Consequently, a wide variety of malignant phenotypes associate with decreased connexin expression and gap junction communication, dependent on the particular connexin that is affected. For instance, deletion of the gene encoding connexin 50, normally expressed in the lens, produces cataracts, though not as severe as with deletion of connexin 46.

REFERENCES

- Manjunath, C.K., Goings, G.E. and Page, E. 1987. Human cardiac gap junctions: isolation, ultrastructure, and protein composition. *J. Mol. Cell. Cardiol.* 19: 131-134.
- Grossman, H.B., Liebert, M., Lee, I.W. and Lee, S.W. 1994. Decreased connexin expression and intercellular communication in human bladder cancer cells. *Cancer Res.* 54: 3062-3065.
- Harris, A.L. 2001. Emerging issues of connexin channels: biophysics fills the gap. *Q. Rev. Biophys.* 34: 325-472.
- Martinez-Wittingham, F.J., Sellitto, C., Li, L., Gong, X., Brink, P.R., Mathias, R.T. and White, T.W. 2003. Dominant cataracts result from incongruous mixing of wildtype lens connexins. *J. Cell Biol.* 161: 969-978.

CHROMOSOMAL LOCATION

Genetic locus: GJA8 (human) mapping to 1q21.2.

PRODUCT

connexin 50 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 connexin 50 shRNA Plasmid (h): sc-43083-SH and connexin 50 shRNA (h) Lentiviral Particles: sc-43083-V as alternate gene silencing products.

For independent verification of connexin 50 (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-43083A, sc-43083B and sc-43083C.

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.

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

connexin 50 siRNA (h) is recommended for the inhibition of connexin 50 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

connexin 50 (B-11): sc-373801 is recommended as a control antibody for monitoring of connexin 50 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 MarkerTM 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 connexin 50 gene expression knockdown using RT-PCR Primer: connexin 50 (h)-PR: sc-43083-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.