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connexin 37 shRNA (h) Lentiviral Particles: sc-43694-V

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 4 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 effected. For example, connexin 37, while expressed in the cardiac endothelium, does not exhibit down-regulation associated with heart failure, as is the case with other cardiac connexins.

REFERENCES

1. Manjunath, C.K., et al. 1987. Human cardiac gap junctions: isolation, ultrastructure, and protein composition. *J. Mol. Cell. Cardiol.* 19: 131-134.
2. Grossman, H.B., et al. 1994. Decreased connexin expression and intercellular communication in human bladder cancer cells. *Cancer Res.* 54: 3062-3065.
3. Boerma, M., et al. 1999. A genetic polymorphism in connexin 37 as a prognostic marker for atherosclerotic plaque development. *J. Intern. Med.* 246: 211-218.
4. Harris AL. 2001. Emerging issues of connexin channels: biophysics fills the gap. *Q. Rev. Biophys.* 34: 325-472.
5. Dupont, E., et al. 2001. Altered connexin expression in human congestive heart failure. *J. Mol. Cell. Cardiol.* 33: 359-371.

CHROMOSOMAL LOCATION

Genetic locus: GJA4 (human) mapping to 1p34.3.

PRODUCT

connexin 37 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see connexin 37 siRNA (h): sc-43694 and connexin 37 shRNA Plasmid (h): sc-43694-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

connexin 37 shRNA (h) Lentiviral Particles is recommended for the inhibition of connexin 37 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

connexin 37 (D-19): sc-27712 is recommended as a control antibody for monitoring of connexin 37 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker[™] compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor connexin 37 gene expression knockdown using RT-PCR Primer: connexin 37 (h)-PR: sc-43694-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.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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