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HCN1 shRNA (h) Lentiviral Particles: sc-44341-V

BACKGROUND

Hyperpolarization-activated, cyclic nucleotide-binding channels (HCN) are voltage-gated cation channels that are activated by direct binding of intracellular cyclic nucleotides. The HCN family consists of four members (HCN1-4), each with a core transmembrane segment domain and a carboxy-terminal 120 amino-acid cyclic nucleotide-binding domain motif. HCN channels are expressed in the brain, heart, thalamus and testis. The pacemaker properties of HCN channels contribute to spontaneous rhythmic activity in the brain and heart. The genes encoding human HCN1 and HCN2 map to chromosomes 5 and 19p13.3, respectively. The genes encoding HCN3 and HCN4 map to chromosomes 1q21.3 and 15q24-q25, respectively.

REFERENCES

1. Vaccari, T., et al. 1999. The human gene coding for HCN2, a pacemaker channel of the heart. *Biochim. Biophys. Acta* 1446: 419-425.
2. Wainger, B.J., et al. 2001. Molecular mechanism of cAMP modulation of HCN pacemaker channels. *Nature* 411: 805-810.
3. Santoro, B., et al. 2004. Regulation of HCN channel surface expression by a novel C-terminal protein-protein interaction. *J. Neurosci.* 24: 10750-10762.
4. Nolan, M.F., et al. 2004. A behavioral role for dendritic integration: HCN1 channels constrain spatial memory and plasticity at inputs to distal dendrites of CA1 pyramidal neurons. *Cell* 119: 719-732.
5. Xiao, J., et al. 2004. Molecular and functional analysis of hyperpolarisation-activated nucleotide-gated (HCN) channels in the enteric nervous system. *Neuroscience* 129: 603-614.
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CHROMOSOMAL LOCATION

Genetic locus: HCN1 (human) mapping to 5p12.

PRODUCT

HCN1 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 HCN1 siRNA (h): sc-44341 and HCN1 shRNA Plasmid (h): sc-44341-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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

HCN1 shRNA (h) Lentiviral Particles is recommended for the inhibition of HCN1 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

HCN1 (V-17): sc-19706 is recommended as a control antibody for monitoring of HCN1 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 HCN1 gene expression knockdown using RT-PCR Primer: HCN1 (h)-PR: sc-44341-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.