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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

HCN3 shRNA (h) Lentiviral Particles: sc-45651-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 C-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. HCN3 contains a segment characterized by a series of positively charged amino acids at every third position. This region designated S4 is likely to be the voltage sensor of the protein. In the brain, HCN3 and HCN4 exhibit subcortical distribution mainly concentrated in the hypothalamus and thalamus, respectively.

REFERENCES

1. Notomi, T., et al. 2004. Immunohistochemical localization of Ih channel subunits, HCN1-4, in the rat brain. *J. Comp. Neurol.* 471: 241-276.
2. Bajorat, R., et al. 2005. Functional significance of HCN2/3-mediated I(h) in striatal cells at early developmental stages. *J. Neurosci. Res.* 82: 206-213.
3. Stieber, J., et al. 2005. Functional expression of the human HCN3 channel. *J. Biol. Chem.* 280: 34635-34643.
4. Varghese, A., et al. 2005. Endogenous channels in HEK cells and potential roles in HCN ionic current measurements. *Prog. Biophys. Mol. Biol.* 90: 26-37
5. Yamada, R., et al. 2005. Hyperpolarization-activated cyclic nucleotide-gated cation channels regulate auditory coincidence detection in nucleus laminaris of the chick. *J. Neurosci.* 25: 8867-8877.
6. SWISS-PROT/TrEMBL (Q9P1Z3). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: HCN3 (human) mapping to 1q22.

PRODUCT

HCN3 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 HCN3 siRNA (h): sc-45651 and HCN3 shRNA Plasmid (h): sc-45651-SH as alternate gene silencing products.

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.

APPLICATIONS

HCN3 shRNA (h) Lentiviral Particles is recommended for the inhibition of HCN3 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

HCN3 (A-3): sc-393813 is recommended as a control antibody for monitoring of HCN3 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 goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HCN3 gene expression knockdown using RT-PCR Primer: HCN3 (h)-PR: sc-45651-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.