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HCN4 siRNA (h): sc-42473

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 1q22 and 15q24.1, respectively.

REFERENCES

1. Ludwig, A., et al. 1999. Two pacemaker channels from human heart with profoundly different activation kinetics. *EMBO J.* 18: 2323-2329.
2. Seifert, R., et al. 1999. Molecular characterization of a slowly gating human hyperpolarization-activated channel predominantly expressed in thalamus, heart, and testis. *Proc. Natl. Acad. Sci. USA* 96: 9391-9396.
3. Vaccari, T., et al. 1999. The human gene coding for HCN2, a pacemaker channel of the heart. *Biochim. Biophys. Acta* 1446: 419-425.
4. Wainger, B.J., et al. 2001. Molecular mechanism of cAMP modulation of HCN pacemaker channels. *Nature* 411: 805-810.
5. Stieber, J., et al. 2003. Molecular basis for the different activation kinetics of the pacemaker channels HCN2 and HCN4. *J. Biol. Chem.* 278: 33672-33680.
6. Stieber, J., et al. 2003. The hyperpolarization-activated channel HCN4 is required for the generation of pacemaker action potentials in the embryonic heart. *Proc. Natl. Acad. Sci. USA* 100: 15235-15240.
7. Ueda, K., et al. 2004. Functional characterization of a trafficking-defective HCN4 mutation, D553N, associated with cardiac arrhythmia. *J. Biol. Chem.* 279: 27194-27198.

CHROMOSOMAL LOCATION

Genetic locus: HCN4 (human) mapping to 15q24.1.

PRODUCT

HCN4 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 HCN4 shRNA Plasmid (h): sc-42473-SH and HCN4 shRNA (h) Lentiviral Particles: sc-42473-V as alternate gene silencing products.

For independent verification of HCN4 (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-42473A, sc-42473B and sc-42473C.

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

HCN4 siRNA (h) is recommended for the inhibition of HCN4 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

HCN4 (SHG 1E5): sc-58622 is recommended as a control antibody for monitoring of HCN4 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HCN4 gene expression knockdown using RT-PCR Primer: HCN4 (h)-PR: sc-42473-PR (20 μ l, 586 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.