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HCN3 siRNA (m): sc-45652

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 Ih 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 (mouse) mapping to 3 F1.

PRODUCT

HCN3 siRNA (m) 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 HCN3 shRNA Plasmid (m): sc-45652-SH and HCN3 shRNA (m) Lentiviral Particles: sc-45652-V as alternate gene silencing products.

For independent verification of HCN3 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45652A, sc-45652B and sc-45652C.

RESEARCH USE

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

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

HCN3 shRNA (m) Lentiviral Particles is recommended for the inhibition of HCN3 expression in mouse 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

HCN3 (E-19): sc-46354 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 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 HCN3 gene expression knockdown using RT-PCR Primer: HCN3 (m)-PR: sc-45652-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.