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Na⁺ CP type III α shRNA (h) Lentiviral Particles: sc-43955-V

BACKGROUND

Voltage-gated sodium channels are selective ion channels that regulate the permeability of sodium ions in excitable cells. During the propagation of an action potential, sodium channels allow an influx of sodium ions, which rapidly depolarize the cell. The three glycoproteins that comprise the voltage-gated sodium channel proteins include a pore-forming α subunit, a noncovalently associated β 1 subunit and a disulfide-linked β 2 subunit. The two β subunits regulate the level of channel expression, modulate gating and function as cell adhesion molecules for cellular aggregation and cytoskeleton interaction. The α subunits of sodium channels type I and III are predominantly expressed in neuronal cell bodies and proximal processes, while type II α subunits are more abundant along axons. The β 1 subunit of sodium channel type I is expressed in brain, skeletal and cardiac muscle. In the brain, β 1 and β 2 are highly expressed in Purkinje cells, and β 1 is also expressed in the pyramidal cells of the deep cerebellar nuclei. Impaired voltage-gated sodium channels lead to a number of diseases including myotonia.

REFERENCES

1. Rosenfeld, J., et al. 1997. A novel muscle sodium channel mutation causes painful congenital myotonia. *Ann. Neurol.* 42: 811-814.
2. Catterall, W.A. 1999. Molecular properties of brain sodium channels: an important target for anticonvulsant drugs. *Adv. Neurol.* 79: 441-456.
3. Whitaker, W.R., et al. 2000. Distribution of voltage-gated sodium channel α -subunit and β -subunit mRNAs in human hippocampal formation, cortex, and cerebellum. *J. Comp. Neurol.* 422: 123-139.
4. Isom, L.L. 2001. Sodium channel β subunits: anything but auxiliary. *Neuroscientist* 7: 42-54.
5. Whitaker, W.R., et al. 2001. Comparative distribution of voltage-gated sodium channel proteins in human brain. *Mol. Brain Res.* 88: 37-53.

CHROMOSOMAL LOCATION

Genetic locus: SCN3A (human) mapping to 2q24.3.

PRODUCT

Na⁺ CP type III α 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 lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Na⁺ CP type III α siRNA (h): sc-43955 and Na⁺ CP type III α shRNA Plasmid (h): sc-43955-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

Na⁺ CP type III α shRNA (h) Lentiviral Particles is recommended for the inhibition of Na⁺ CP type III α 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 lentiviral transducing particles per milliliter; contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Na⁺ CP type III α (S-15): sc-22202 is recommended as a control antibody for monitoring of Na⁺ CP type III α 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 Na⁺ CP type III α gene expression knockdown using RT-PCR Primer: Na⁺ CP type III α (h)-PR: sc-43955-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.

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.

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