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NCX1 shRNA (m) Lentiviral Particles: sc-44515-V

BACKGROUND

Sodium/calcium exchanger proteins are integral membrane proteins primarily seen in cardiac cells. In cardiac myocytes, the concentration of Ca^{2+} alternates between low levels during relaxation and high levels during contraction. The Na^+/Ca^{2+} exchanger 1 (NCX1) protein mediates Ca^{2+} extrusion from cardiac cells during relaxation. Four NCX1 isoforms (NCX1.1, NCX1.3, NCX1.7, and NCX1.10) result from alternate splicing. NCX1 mRNA is present at high levels in the heart, with lower levels present in the brain. NCX2 is most abundantly expressed in brain, in contrast the the broader distribution of NCX1, which is also expressed in heart, kidney, lung, smooth and skeletal muscle. The difference in expression for the transporter subtypes is believed to reflect differences in their functional roles. Regulation mechanisms for these exchanger proteins have not been fully characterized.

REFERENCES

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- Frayse, B., et al. 2001. Expression of the Na^+/Ca^{2+} exchanger in skeletal muscle. *Am. J. Physiol., Cell Physiol.* 280: C146-154.
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- Thurneysen, T., et al. 2002. Sodium/calcium exchanger subtypes NCX1, NCX2 and NCX3 show cell-specific expression in rat hippocampus cultures. *Brain Res. Mol. Brain Res.* 107: 145-156.

CHROMOSOMAL LOCATION

Genetic locus: Slc8a1 (mouse) mapping to 17 E3.

PRODUCT

NCX1 shRNA (m) 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 NCX1 siRNA (m): sc-44515 and NCX1 shRNA Plasmid (m): sc-44515-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

NCX1 shRNA (m) Lentiviral Particles is recommended for the inhibition of NCX1 expression in mouse 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

NCX1 (P-13)-R: sc-30304-R is recommended as a control antibody for monitoring of NCX1 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NCX1 gene expression knockdown using RT-PCR Primer: NCX1 (m)-PR: sc-44515-PR (20 μ l, 449 bp). 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.

PROTOCOLS

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