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NCX3 shRNA (m) Lentiviral Particles: sc-44911-V

BACKGROUND

Sodium/calcium exchanger proteins are integral membrane proteins primarily seen in cardiac cells. In cardiac myocytes, the concentration of Ca²⁺ alternates between low levels during relaxation and high levels during contraction. NCX3 (Na⁺/Ca²⁺-exchange protein 3), also known as SLC8A3 (solute carrier family 8 (sodium/calcium exchanger), member 3), is a 927 amino acid multi-pass membrane protein belonging to the sodium/potassium/calcium exchanger family. Expressed as three alternatively spliced isoforms, NCX3 rapidly transports Ca²⁺ during excitation-contraction coupling. Containing two Calx- β domains, NCX3 is expressed in brain, skeletal muscle and retina. As a major functional sodium-calcium exchanger in osteoblasts, NCX3 is involved in the translocation of Ca²⁺ out of osteoblasts into calcifying bone matrix.

REFERENCES

- Gabellini, N., et al. 2002. The human SLC8A3 gene and the tissue-specific Na⁺/Ca²⁺ exchanger 3 isoforms. *Gene* 298: 1-7.
- Gabellini, N., et al. 2003. Control of the Na⁺/Ca²⁺ exchanger 3 promoter by cyclic adenosine monophosphate and Ca²⁺ in differentiating neurons. *J. Neurochem.* 84: 282-293.
- Bojarski, C., et al. 2008. Na⁺/Ca²⁺ exchanger subtype (NCX1, NCX2, NCX3) protein expression in the rat hippocampus following 3 min and 8 min durations of global cerebral ischemia. *Brain Res.* 1189: 198-202.
- Wu, M.P., et al. 2008. Reverse mode Na⁺/Ca²⁺ exchangers trigger the release of Ca²⁺ from intracellular Ca²⁺ stores in cultured rat embryonic cortical neurons. *Brain Res.* 1201: 41-51.
- Sosnoski, D.M. and Gay, C.V. 2008. NCX3 is a major functional isoform of the sodium-calcium exchanger in osteoblasts. *J. Cell. Biochem.* 103: 1101-1110.
- Molinario, P., et al. 2008. Targeted disruption of Na⁺/Ca²⁺ exchanger 3 (NCX3) gene leads to a worsening of ischemic brain damage. *J. Neurosci.* 28: 1179-1184.

CHROMOSOMAL LOCATION

Genetic locus: Slc8a3 (mouse) mapping to 12 D1.

PRODUCT

NCX3 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 x 10⁶ 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 NCX3 siRNA (m): sc-44911 and NCX3 shRNA Plasmid (m): sc-44911-SH as alternate gene silencing products.

APPLICATIONS

NCX3 shRNA (m) Lentiviral Particles is recommended for the inhibition of NCX3 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 10⁶ 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

NCX3 (C-15): sc-48896 is recommended as a control antibody for monitoring of NCX3 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 NCX3 gene expression knockdown using RT-PCR Primer: NCX3 (m)-PR: sc-44911-PR (20 μ l, 593 bp). 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.

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.