SANTA CRUZ BIOTECHNOLOGY, INC.

NCX2 siRNA (h): sc-44908

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. Na^{+}/Ca^{2+} exchanger 2 (NCX2) is most abundantly expressed in brain. This is in contrast to 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

1. Li, Z., et al. 1994. Cloning of the NCX2 isoform of the plasma membrane Na^{+}/Ca^{2+} exchanger. J. Biol. Chem. 269: 17434-17439.
2. Kikuno, R., et al. 1999. Prediction of the coding sequences of unidentified human genes. XIV. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. DNA Res. 6: 197-205.
3. Li, L., et al. 2000. Calcineurin controls the transcription of Na^{+}/Ca^{2+} exchanger isoforms in developing cerebellar neurons. J. Biol. Chem. 275: 20903-20910.
4. Fraysse, B., et al. 2001. Expression of the Na^{+}/Ca^{2+} exchanger in skeletal muscle. Am. J. Physiol. Cell Physiol. 280: C146-C154.
5. Canitano, A., et al. 2002. Brain distribution of the Na^{+}/Ca^{2+} exchanger-encoding genes NCX1, NCX2 and NCX3 and their related proteins in the central nervous system. Ann. N.Y. Acad. Sci. 976394-976404.
6. 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.
7. Papa, M., et al. 2003. Differential expression of the Na^{+}/Ca^{2+} exchanger transcripts and proteins in rat brain regions. J. Comp. Neurol. 461: 31-48.
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CHROMOSOMAL LOCATION

Genetic locus: SLC8A2 (human) mapping to 19q13.32.

PRODUCT

NCX2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NCX2 shRNA Plasmid (h): sc-44908-SH and NCX2 shRNA (h) Lentiviral Particles: sc-44908-V as alternate gene silencing products.

For independent verification of NCX2 (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-44908A, sc-44908B and sc-44908C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NCX2 siRNA (h) is recommended for the inhibition of NCX2 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 µM in 66 µl. 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

NCX2 (E-1): sc-515768 is recommended as a control antibody for monitoring of NCX2 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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NCX2 gene expression knockdown using RT-PCR Primer: NCX2 (h)-PR: sc-44908-PR (20 µl, 600 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.