SANTA CRUZ BIOTECHNOLOGY, INC.

NCKX2 siRNA (h): sc-72258

BACKGROUND

NCKX2, also designated solute carrier family 24, member 2 (SLC24A2), belongs to a family of potassium-dependent sodium/calcium exchangers, all of which contain two large hydrophilic loops and two sets of multiple transmembrane-spanning segments. NCKX2 is expressed in neurons throughout the brain, with high expression found in cone photoreceptors in the retina as well as hippocampal pyramidal cells and the deep nuclei of the cerebellum. A critical component in the process of light adaptation, NCKX2 transports one calcium ion and one potassium ion in exchange for four sodium ions, thereby contolling calcium concentration in the eye during light and darkness. Additionally, NCKX2 regulates calcium levels in other cellular locations that experience rapid calcium fluxes, such as neuronal synapses. Inactivation of NCKX2 is believed to be an important regulatory mechanism in the control of sodium and calcium levels under different physiological conditions.

REFERENCES

1. Tsoi, M., et al. 1998. Molecular cloning of a novel potassium-dependent sodium-calcium exchanger from rat brain. J. Biol. Chem. 273: 4155-4162.
2. Li, X.F., et al. 2002. Molecular cloning of a fourth member of the potassium-dependent sodium-calcium exchanger gene family, NCKX4. J. Biol. Chem. 277: 48410-48417.
3. Cai, X., et al. 2002. A novel topology and redox regulation of the rat brain K^+-dependent Na^+/Ca^{2+} exchanger, NCKX2. J. Biol. Chem. 277: 48923-48930.
4. Kang, K.J., et al. 2005. Residues contributing to the Ca^{2+} and K^+ binding pocket of the NCKX2 Na^+/Ca^{2+}-K^+ exchanger. J. Biol. Chem. 280: 6823-6833.
5. Kang, K.J., et al. 2005. Substitution of a single residue, Asp575, renders the NCKX2 K^+-dependent Na^+/Ca^{2+} exchanger independent of K^+. J. Biol. Chem. 280: 6834-6839.
6. Kinjo, T.G., et al. 2005. Site-directed disulfide mapping of residues contributing to the Ca^{2+} and K^+ binding pocket of the NCKX2 Na^+/Ca^{2+}-K^+ exchanger. Biochemistry 44: 7787-7795.

CHROMOSOMAL LOCATION

Genetic locus: SLC24A2 (human) mapping to 9p22.1.

PRODUCT

NCKX2 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 NCKX2 shRNA Plasmid (h): sc-72258-SH and NCKX2 shRNA (h) Lentiviral Particles: sc-72258-V as alternate gene silencing products.

For independent verification of NCKX2 (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-72258A, sc-72258B and sc-72258C.

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

NCKX2 siRNA (h) is recommended for the inhibition of NCKX2 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

NCKX2 (F-2): sc-514412 is recommended as a control antibody for monitoring of NCKX2 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 NCKX2 gene expression knockdown using RT-PCR Primer: NCKX2 (h)-PR: sc-72258-PR (20 µl). 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.