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NHE-6 siRNA (h): sc-42658

BACKGROUND

Na⁺/H⁺ exchangers-1-6 (Na⁺/H⁺ antiporters, NHE-1-6) are integral membrane proteins that are expressed in most mammalian tissues where they regulate intracellular pH and cell volume. NHE's mediate the secondary active extrusion of hydrogen (H⁺) ions out of cells in exchange for extracellular sodium (Na⁺). Excluding NHE-1, which is ubiquitously expressed, the NHE isoforms 2-6 have distinct tissue- and cell type-dependent expression, and inhibitory characteristics by amiloride analogs.

REFERENCES

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2. Biemesderfer, D., et al. 1993. NHE3: a Na⁺/H⁺ exchanger isoform of renal brush border. *Am. J. Physiol.* 265: 736-742.
3. Klanke, C.A., et al. 1995. Molecular cloning and physical and genetic mapping of a novel human Na⁺/H⁺ exchanger (NHE5/SLC9A5) to chromosome 16q22.1. *Genomics* 25: 615-622.
4. Noel, J., et al. 1995. Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na⁺/H⁺ exchanger isoforms. *Am. J. Physiol.* 268: 283-296.
5. Cox, G.A., et al. 1997. Sodium/hydrogen exchanger gene defect in slow-wave epilepsy mutant mice. *Cell* 91: 139-148.
6. Baird, N.R., et al. 1999. Molecular cloning, genomic organization, and functional expression of Na⁺/H⁺ exchanger isoform 5 (NHE5) from human brain. *J. Biol. Chem.* 274: 4377-4382.

CHROMOSOMAL LOCATION

Genetic locus: SLC9A6 (human) mapping to Xq26.3.

PRODUCT

NHE-6 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 NHE-6 shRNA Plasmid (h): sc-42658-SH and NHE-6 shRNA (h) Lentiviral Particles: sc-42658-V as alternate gene silencing products.

For independent verification of NHE-6 (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-42658A, sc-42658B and sc-42658C.

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

NHE-6 siRNA (h) is recommended for the inhibition of NHE-6 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

NHE-6 (2D5): sc-517111 is recommended as a control antibody for monitoring of NHE-6 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 NHE-6 gene expression knockdown using RT-PCR Primer: NHE-6 (h)-PR: sc-42658-PR (20 μl, 539 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.