

NHE-5 siRNA (h): sc-42656

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. NHEs 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, NHE isoforms 2–6 have distinct tissue- and cell type-dependent expression and inhibitory characteristics by amiloride analogs. Human NHE-5 transcript (solute carrier family 9, isoform-5, SLC9A5) is an 896 amino acid protein that is expressed in brain, testis, spleen, and skeletal muscle.

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. Noel, J., et al. 1995. Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na⁺/H⁺ exchanger isoforms. *Am. J. Physiol.* 268: 283–296.
4. 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.
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: SLC9A5 (human) mapping to 16q22.1.

PRODUCT

NHE-5 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 μ M solution once resuspended using protocol below. Suitable for 50–100 transfections. Also see NHE-5 shRNA Plasmid (h): sc-42656-SH and NHE-5 shRNA (h) Lentiviral Particles: sc-42656-V as alternate gene silencing products.

For independent verification of NHE-5 (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-42656A, sc-42656B and sc-42656C.

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.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NHE-5 siRNA (h) is recommended for the inhibition of NHE-5 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 μ M in 66 μ l. 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NHE-5 gene expression knockdown using RT-PCR Primer: NHE-5 (h)-PR: sc-42656-PR (20 μ l, 589 bp). Annealing temperature for the primers should be 55–60° C and the extension temperature should be 68–72° C.