NHE-1 siRNA (m): sc-42651



The Power to Question

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, the NHE isoforms NHE-2-6 have distinct tissue- and cell type-dependent expression and inhibitory characteristics by amiloride analogs. Human NHE-1 protein, known also as solute carrier family 9 isoform-1 (SLC9A1), is a ten transmembrane domain-spanning receptor that contains an N-terminal amphiphatic domain and two putative N-glycosylation sites.

REFERENCES

- Sardet, C., et al. 1989. Molecular cloning, primary structure, and expression of the human growth factor-activatable Na+/H+ antiporter. Cell 56: 271-280.
- Orlowski, J., et al. 1992. Molecular cloning of putative members of the Na/H exchanger gene family. cDNA cloning, deduced amino acid sequence, and mRNA tissue expression of the rat Na/H exchanger NHE-1 and two structurally related proteins. J. Biol. Chem. 267: 9331-9339.
- 3. Fliegel, L., et al. 1993. Cloning and analysis of the human myocardial Na+/H+ exchanger. Mol. Cell. Biochem. 125: 137-143.
- 4. Biemesderfer, D., et al. 1993. NHE3: a Na+/H+ exchanger isoform of renal brush border. Am. J. Physiol. 265: 736-742.
- Noel, J., et al. 1995. Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na+/H+ exchanger isoforms. Am. J. Physiol. 268: 283-296.
- 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.
- Cox, G.A., et al. 1997. Sodium/hydrogen exchanger gene defect in slowwave epilepsy mutant mice. Cell 91: 139-148.
- 8. Denker, S.P., et al. 2001. Direct binding of the Na—H exchanger NHE1 to ERM proteins regulates the cortical cytoskeleton and cell shape independently of H+ translocation. Mol. Cell 6: 1425-1436.

CHROMOSOMAL LOCATION

Genetic locus: Slc9a1 (mouse) mapping to 4 D2.3.

PRODUCT

NHE-1 siRNA (m) 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-1 shRNA Plasmid (m): sc-42651-SH and NHE-1 shRNA (m) Lentiviral Particles: sc-42651-V as alternate gene silencing products.

For independent verification of NHE-1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-42651A, sc-42651B and sc-42651C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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-1 siRNA (m) is recommended for the inhibition of NHE-1 expression in mouse 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-1 (54): sc-136239 is recommended as a control antibody for monitoring of NHE-1 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NHE-1 gene expression knockdown using RT-PCR Primer: NHE-1 (m)-PR: sc-42651-PR (20 μ l, 415 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.

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