

NHE-1 siRNA (h): sc-42650

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 amphipathic domain and two putative N-glycosylation sites.

REFERENCES

1. Sardet, C., et al. 1989. Molecular cloning, primary structure, and expression of the human growth factor-activatable Na⁺/H⁺ antiporter. *Cell* 56: 271-280.
2. Orłowski, 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. NHE-3: a Na⁺/H⁺ exchanger isoform of renal brush border. *Am. J. Physiol.* 265: 736-742.
5. Noel, J. and Pouyssegur, J. 1995. Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na⁺/H⁺ exchanger isoforms. *Am. J. Physiol.* 268: 283-296.

CHROMOSOMAL LOCATION

Genetic locus: SLC9A1 (human) mapping to 1p36.11.

PRODUCT

NHE-1 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-1 shRNA Plasmid (h): sc-42650-SH and NHE-1 shRNA (h) Lentiviral Particles: sc-42650-V as alternate gene silencing products.

For independent verification of NHE-1 (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-42650A, sc-42650B and sc-42650C.

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-1 siRNA (h) is recommended for the inhibition of NHE-1 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-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).

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-1 gene expression knockdown using RT-PCR Primer: NHE-1 (h)-PR: sc-42650-PR (20 μl, 497 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Bocanegra, V., et al. 2014. RhoA and MAPK signal transduction pathways regulate NHE1-dependent proximal tubule cell apoptosis after mechanical stretch. *Am. J. Physiol. Renal Physiol.* 307: F881-F889.
2. Sanhueza, C., et al. 2017. Sodium/proton exchanger isoform 1 regulates intracellular pH and cell proliferation in human ovarian cancer. *Biochim. Biophys. Acta Mol. Basis Dis.* 1863: 81-91.
3. Son, A., et al. 2022. Targeting Na-H exchanger 1 overcomes nuclear factor κB-mediated tumor resistance to radiotherapy. *Neoplasia* 35: 100862.

RESEARCH USE

For research use only, not for use in diagnostic procedures.