

NHE-3 siRNA (m): sc-36060

BACKGROUND

Na⁺/H⁺ exchangers-1-8 (also designated NHE-1-8 or Na⁺/H⁺ antiporters) are integral membrane proteins that are expressed in most mammalian tissues, where they regulate intracellular pH and cell volume. NHEs mediate the transport of hydrogen (H⁺) ions out of cells in exchange for extracellular sodium (Na⁺) ions. While NHE-1 is ubiquitously expressed, the NHE isoforms 2-8 have distinct tissue- and cell type-dependent expression and inhibitory characteristics. NHE-3 localizes to the apical membrane of renal proximal tubules where it is responsible for most of the sodium transport and fluid reabsorption. NHE-3 translocates to internal pools where it mediates natriuresis when blood pressure increases abruptly. NHE-3 is also expressed in the stomach and functions to protect the mucosa by secreting protons that diffuse into the mucous cells.

REFERENCES

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- Harris, S.P., et al. 1997. Epithelial localization of a reptilian Na⁺/H⁺ exchanger homologous to NHE-1. *Am. J. Physiol.* 272: 1594-1606.
- Kulaksiz, H., et al. 2001. Expression and cell-specific and membrane-specific localization of NHE-3 in the human and guinea pig upper gastrointestinal tract. *Cell Tissue Res.* 303: 337-343.
- LaPointe, M.S., et al. 2002. Na⁺/H⁺ exchange activity and NHE-3 expression in renal tubules from the spontaneously hypertensive rat. *Kidney Int.* 62: 157-165.
- Sangan, P., et al. 2002. Cloning and expression of a chloride-dependent Na⁺/H⁺ exchanger. *J. Biol. Chem.* 277: 9668-9675.
- Goyal, S., et al. 2003. Renal expression of novel Na⁺/H⁺ exchanger isoform NHE-8. *Am. J. Physiol. Renal Physiol.* 284: 467-473.
- Goyal, S., et al. 2005. Immunolocalization of NHE-8 in rat kidney. *Am. J. Physiol. Renal Physiol.* 288: 530-538.

CHROMOSOMAL LOCATION

Genetic locus: Slc9a3 (mouse) mapping to 13 C1.

PRODUCT

NHE-3 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-3 shRNA Plasmid (m): sc-36060-SH and NHE-3 shRNA (m) Lentiviral Particles: sc-36060-V as alternate gene silencing products.

For independent verification of NHE-3 (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-36060A, sc-36060B and sc-36060C.

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-3 siRNA (m) is recommended for the inhibition of NHE-3 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

p-NHE-3 (14D5): sc-53962 is recommended as a control antibody for monitoring of NHE-3 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, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Lambda Phosphatase: sc-200312A 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-3 gene expression knockdown using RT-PCR Primer: NHE-3 (m)-PR: sc-36060-PR (20 μl, 568 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.