

NHE-8 siRNA (h): sc-75912

BACKGROUND

Na⁺/H⁺ exchangers 1-8, also designated Na⁺/H⁺ antiporters or NHE-1-8, are integral membrane proteins 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, NHE isoforms 2-8 have distinct tissue and cell type dependent expression and inhibitory characteristics. NHE-8 is a 575 amino acid protein that localizes apically in intestinal epithelial cells. Expression of NHE-8 is higher in young mammals than adults. NHE-8 gene and protein expression are highly regulated during ontogeny; this protein may play an important role in intestinal Na⁺ absorption during early mammalian life.

REFERENCES

1. 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.
2. Harris, S.P., et al. 1997. Epithelial localization of a reptilian Na⁺/H⁺ exchanger homologous to NHE-1. *Am. J. Physiol.* 272: G1594-G1606.
3. Sangan, P., et al. 2002. Cloning and expression of a chloride-dependent Na⁺/H⁺ exchanger. *J. Biol. Chem.* 277: 9668-9675.
4. Goyal, S., et al. 2003. Renal expression of novel Na⁺/H⁺ exchanger isoform NHE8. *Am. J. Physiol. Renal Physiol.* 284: F467-F473.
5. Goyal, S., et al. 2005. Immunolocalization of NHE8 in rat kidney. *Am. J. Physiol. Renal Physiol.* 288: F530-F538.
6. Xu, H., et al. 2005. Subcloning, localization, and expression of the rat intestinal sodium-hydrogen exchanger isoform 8. *Am. J. Physiol. Gastrointest. Liver Physiol.* 289: G36-G41.

CHROMOSOMAL LOCATION

Genetic locus: SLC9A8 (human) mapping to 20q13.13.

PRODUCT

NHE-8 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-8 shRNA Plasmid (h): sc-75912-SH and NHE-8 shRNA (h) Lentiviral Particles: sc-75912-V as alternate gene silencing products.

For independent verification of NHE-8 (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-75912A, sc-75912B and sc-75912C.

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-8 siRNA (h) is recommended for the inhibition of NHE-8 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.

GENE EXPRESSION MONITORING

NHE-8 (7A11): sc-53902 is recommended as a control antibody for monitoring of NHE-8 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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-8 gene expression knockdown using RT-PCR Primer: NHE-8 (h)-PR: sc-75912-PR (20 μ l, 541 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.