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SANTA CRUZ BIOTECHNOLOGY, INC.

NHE-8 siRNA (m): sc-149957

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: 1594-1606.
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 NHE-8. Am. J. Physiol. Renal Physiol. 284: 467-473.
5. Goyal, S., et al. 2005. Immunolocalization of NHE-8 in rat kidney. Am. J. Physiol. Renal Physiol. 288: 530-538.
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: 36-41.

CHROMOSOMAL LOCATION

Genetic locus: Slc9a8 (mouse) mapping to 2 H3.

PRODUCT

NHE-8 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-8 shRNA Plasmid (m): sc-149957-SH and NHE-8 shRNA (m) Lentiviral Particles: sc-149957-V as alternate gene silencing products.

For independent verification of NHE-8 (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-149957A, sc-149957B and sc-149957C.

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 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-8 siRNA (m) is recommended for the inhibition of NHE-8 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-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-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-8 gene expression knockdown using RT-PCR Primer: NHE-8 (m)-PR: sc-149957-PR (20 µl, 550 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.