

# NHERF-1 siRNA (h): sc-63330

## BACKGROUND

The Na<sup>+</sup>/H<sup>+</sup> exchange protein (NHE3) functions in transepithelial Na<sup>+</sup> absorption and is primarily expressed in the intestinal and renal brush border membrane. NHE3 regulatory factor 1 (NHERF-1) interacts with NHE3 through two PDZ (for PSD-95, Discs-large and ZO-1 homology) domains, which are protein-protein interaction modules that associate with specific carboxy-terminal motifs on target proteins. Also known as EBP50, NHERF-1 facilitates cAMP inhibition of NHE3 to decrease Na<sup>+</sup> adsorption. NHERF-1 functions as a scaffold for an essential multiprotein complex of Ezrin and NHE3 for cAMP-mediated phosphorylation and consequent inhibition of NHE3. The amino-terminal PDZ domain regulates the dimerization of NHERF-1 *in vivo*. G protein-coupled receptor kinase 6A phosphorylates NHERF-1 at Ser 289 via a PDZ domain-mediated interaction. NHERF-2, also known as E3KARP, is an ubiquitously expressed protein which also functions in NHE2 regulation.

## REFERENCES

1. Aronson, P.S., et al. 1986. Molecular-properties and physiological roles of the renal Na<sup>+</sup>-H<sup>+</sup> exchanger. *Curr. Top. Mem. Trans.* 26: 57-75.
2. Sheng, M. 1996. PDZs and receptor/channel clustering: rounding up the latest suspects. *Neuron* 17: 575-578.
3. Yun, C.H., et al. 1997. cAMP-mediated inhibition of the epithelial brush border Na<sup>+</sup>/H<sup>+</sup> exchanger, NHE3, requires an associated regulatory protein. *Proc. Natl. Acad. Sci. USA* 94: 3010-3015.
4. Poulat, F., et al. 1997. The human testis determining factor SRY binds a nuclear factor containing PDZ protein interaction domains. *J. Biol. Chem.* 272: 7167-7172.

## CHROMOSOMAL LOCATION

Genetic locus: SLC9A3R1 (human) mapping to 17q25.1.

## PRODUCT

NHERF-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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NHERF-1 shRNA Plasmid (h): sc-63330-SH and NHERF-1 shRNA (h) Lentiviral Particles: sc-63330-V as alternate gene silencing products.

For independent verification of NHERF-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-63330A, sc-63330B and sc-63330C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

NHERF-1 siRNA (h) is recommended for the inhibition of NHERF-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  $\mu$ M in 66  $\mu$ 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

NHERF-1 (A-7): sc-271552 is recommended as a control antibody for monitoring of NHERF-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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NHERF-1 gene expression knockdown using RT-PCR Primer: NHERF-1 (h)-PR: sc-63330-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Ji, M.Y., et al. 2012. The detection of EBP50 expression using quantum dot immunohistochemistry in pancreatic cancer tissue and down-regulated EBP50 effect on PC-2 cells. *J. Mol. Histol.* 43: 517-526.
2. Liu, H., et al. 2015. SLC9A3R1 stimulates autophagy via BECN1 stabilization in breast cancer cells. *Autophagy* 11: 2323-2334.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.