

NHERF-1 siRNA (r): sc-156113

BACKGROUND

The Na⁺/H⁺ exchange protein (NHE3) functions in transepithelial Na⁺ absorption and is primarily expressed in the intestinal and renal brush border membrane. NHERF 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⁺ 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

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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⁺/H⁺ 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.
5. Imai, K., et al. 1998. Genomic structure and sequence of a human homologue (NTHL1/NTH1) of *Escherichia coli* endonuclease III with those of the adjacent parts of TSC2 and SLC9A3R2 genes. *Gene* 222: 287-295.
6. Hall, R.A., et al. 1999. G protein-coupled receptor kinase 6A phosphorylates the Na⁺/H⁺ exchanger regulatory factor via a PDZ domain-mediated interaction. *J. Biol. Chem.* 274: 24328-24334.
7. Weinman, E.J., et al. 2000. NHERF associations with sodium-hydrogen exchanger isoform 3 (NHE3) and Ezrin are essential for cAMP-mediated phosphorylation and inhibition of NHE3. *Biochem.* 39: 6123-6129.
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CHROMOSOMAL LOCATION

Genetic locus: Slc9a3r1 (rat) mapping to 10q32.2.

PRODUCT

NHERF-1 siRNA (r) is a target-specific 19-25 nt siRNA 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 NHERF-1 shRNA Plasmid (r): sc-156113-SH and NHERF-1 shRNA (r) Lentiviral Particles: sc-156113-V as alternate gene silencing 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

NHERF-1 siRNA (r) is recommended for the inhibition of NHERF-1 expression in rat 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

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 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 NHERF-1 gene expression knockdown using RT-PCR Primer: NHERF-1 (r)-PR: sc-156113-PR (20 μ l). 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.