

EPI64 siRNA (h): sc-42520

BACKGROUND

The Na⁺/H⁺ exchange protein (NHE) functions in transepithelial Na⁺ absorption and is primarily expressed in the intestinal and renal brush border membrane. NHE regulatory factor 1 (NHERF-1) interacts with NHE through two PDZ (for PSD-95, discs-large, and ZO-1 homology) domains, which are protein-protein interaction modules that associate with specific C-terminal motifs on target proteins. Also known as EBP50, NHERF-1 facilitates cAMP inhibition of NHE to decrease Na⁺ adsorption. NHERF-2, also known as E3KARP, is ubiquitously expressed as a protein which also functions in NHE2 regulation. EPI64 (EBP-PDZ interactor) contains a C-terminal -DTYL sequence that binds to the first PDZ domain of NHERF-1 and NHERF-2. EPI64 is ubiquitously expressed and localizes with NHERF-1 *in vitro*.

REFERENCES

- Sheng, M. 1996. PDZs and receptor/channel clustering: rounding up the latest suspects. *Neuron* 17: 575-578.
- 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.
- Poulat, F., et al. 1997. The human testis determining factor SRY bind a nuclear factor containing PDZ protein interaction domains. *J. Biol. Chem.* 272: 7167-7172.
- Reczek, D. and Bretscher, A. 2001. Identification of EPI64, a TBC/rabGAP domain-containing microvillar protein that binds to the first PDZ domain of EBP50 and E3KARP. *J. Cell Biol.* 153: 191-205.
- Itoh, T. and Fukuda, M. 2006. Identification of EPI64 as a GTPase-activating protein specific for Rab27A. *J. Biol. Chem.* 281: 31823-31831.
- Hanono, A., et al. 2006. EPI64 regulates microvillar subdomains and structure. *J. Cell Biol.* 175: 803-813.

CHROMOSOMAL LOCATION

Genetic locus: TBC1D10A (human) mapping to 22q12.2.

PRODUCT

EPI64 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 EPI64 shRNA Plasmid (h): sc-42520-SH and EPI64 shRNA (h) Lentiviral Particles: sc-42520-V as alternate gene silencing products.

For independent verification of EPI64 (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-42520A, sc-42520B and sc-42520C.

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

EPI64 siRNA (h) is recommended for the inhibition of EPI64 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

EPI64 (B-9): sc-376991 is recommended as a control antibody for monitoring of EPI64 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 EPI64 gene expression knockdown using RT-PCR Primer: EPI64 (h)-PR: sc-42520-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.