SANTA CRUZ BIOTECHNOLOGY, INC.

αENaC siRNA (h): sc-42404

BACKGROUND

The epithelial sodium channel (ENaC) is a member of the ENaC/DEG superfamily that is located on the apical surface of cells. ENaC mediates sodium reabsorption in kidney, distal colon, lung, ducts of exocrine glands and other organs. ENaC is formed by heteromultimerization of four homologous subunits, α, β, γ and δ. The most frequently formed heterotetramer consists of 2α, 1β and 1γ subunit, but the α subunit can be replaced by a δ subunit. The αENaC gene maps to human chromosome 12p13. Both the β and γENaC genes map to human chromosome 16p12 and the γENaC transcript is detected as a glycosylated protein. The carboxy-terminus of all ENaC subunits contains PY motifs, which interact with the ubiquitin protein ligase, Nedd4, to regulate intracellular sodium concentrations. Gain-of-function mutations involving the PY motif cause Liddle's syndrome, an autosomal dominant form of hypertension, resulting from excessive renal sodium absorption. Conversely, ENaC loss-of-function mutations result in pseudohypoaldosteronism type I, a disorder characterized by salt wasting and hypotension.

REFERENCES

1. McDonald, F.J., et al. 1994. Cloning, expression, and tissue distribution of a human amiloride-sensitive Na^+ channel. Am. J. Physiol. 266: L728-L734.
2. Voilley, N., et al. 1995. Cloning, chromosomal localization, and physical linkage of the β and γ subunits (SCNN1B and SCNN1G) of the human epithelial amiloride-sensitive sodium channel. Genomics 28: 560-565.
3. Ludwig, M., et al. 1998. Structural organization of the gene encoding the α subunit of the human amiloride-sensitive epithelial sodium channel. Hum. Genet. 102: 576-581.
4. Masilamani, S., et al. 1999. Aldosterone-mediated regulation of ENaC α, β, and γ subunit proteins in rat kidney. J. Clin. Invest. 104: R19-R23.
5. Hanwell, D., et al. 2002. Trafficking and cell surface stability of the epithelial Na^+ channel expressed in epithelial Madin-Darby canine kidney cells. J. Biol. Chem. 277: 9772-9779.
6. Brockway, L.M., et al. 2002. Rabbit retinal neurons and glia express a variety of ENaC/DEG subunits. Am. J. Physiol., Cell Physiol. 283: C126-C134.
7. Snyder, P.M. 2002. The epithelial Na^+ channel: cell surface insertion and retrieval in Na^+ homeostasis and hypertension. Endocr. Rev. 23: 258-275.

CHROMOSOMAL LOCATION

Genetic locus: SCNN1A (human) mapping to 12p13.31.

PRODUCT

αENaC 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see αENaC shRNA Plasmid (h): sc-42404-SH and αENaC shRNA (h) Lentiviral Particles: sc-42404-V as alternate gene silencing products.

For independent verification of αENaC (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-42404A, sc-42404B and sc-42404C.

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

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

αENaC (H-6): sc-518119 is recommended as a control antibody for monitoring of αENaC 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 αENaC gene expression knockdown using RT-PCR Primer: αENaC (h)-PR: sc-42404-PR (20 µl). 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.