SANTA CRUZ BIOTECHNOLOGY, INC.

Barttin siRNA (h): sc-60245

BACKGROUND

The BSND gene encodes Barttin, a protein comprised of two putative transmembrane α helices. Barttin expression is detected in the thin limb and thick ascending limb of the loop of Henle in the kidney, and in the dark cells of the inner ear. The BSND gene is mutated in Bartter syndrome, a genetic disease characterized by hypokalemia, metabolic alkalosis and normal to low blood pressure. This occurs with sensorineural deafness; irreversible hearing loss due to cochlear sensorineural or cochlear nerve damage. Barttin acts as an essential β subunit for CLCKNA and CLCKNB chloride channels, with which it co-localizes in basolateral membranes of renal tubules and of potassium-secreting epithelia of the inner ear. Mutations in either CLCKNB or Barttin compromise currents through heteromeric channels that can be stimulated further by mutating a proline-tyrosine (PY) motif on Barttin. Heteromers formed by chloride channels and Barttin are essential for renal salt reabsorption and potassium recycling in the inner ear.

REFERENCES

1. Estevez, R., et al. 2001. Barttin is a Cl- channel β subunit crucial for renal Cl^- reabsorption and inner ear K^+ secretion. Nature 414: 558-561.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606412. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Miyamura, N., et al. 2003. Atypical Bartter syndrome with sensorineural deafness with G47R mutation of the β subunit for ClC-Ka and ClC-Kb chloride channels, Barttin. J. Clin. Endocrinol. Metab. 88: 781-786.
4. Wolf, K., et al. 2003. Parallel downregulation of chloride channel CLC-K1 and Barttin mRNA in the thin ascending limb of the rat nephron by furosemide. Pflugers Arch. 446: 665-671.

CHROMOSOMAL LOCATION

Genetic locus: BSND (human) mapping to 1p32.3.

PRODUCT

Barttin 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 Barttin shRNA Plasmid (h): sc-60245-SH and Barttin shRNA (h) Lentiviral Particles: sc-60245-V as alternate gene silencing products.

For independent verification of Barttin (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-60245A, sc-60245B and sc-60245C.

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

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

Barttin (A-1): sc-271867 is recommended as a control antibody for monitoring of Barttin 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 Barttin gene expression knockdown using RT-PCR Primer: Barttin (h)-PR: sc-60245-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.