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SANTA CRUZ BIOTECHNOLOGY, INC.

ROM-K siRNA (h): sc-42632

BACKGROUND

ROM-K, an ATP-sensitive inward rectifying K^+ channel (also designated KIR1.1), is a member of the Kir family of K^+ channels that controls renal K^+ secretion. These K^+ channels more readily conduct an inward current rather than an outward current and are constituitively open. Inwardly rectifying K^+ channels are a complex of four Kir (Kir1-6) subunits. ROM-K is activated by protein kinase A, and its activity is regulated by phosphatidylinositol 4,5-bisphosphate and intracellular pH. Alternative splicing of ROM-K mRNA yields various isoforms which are differentially expressed in nephrons of the mammalian kidney. Mutations in the ROM-K gene are linked to antenatal Bartter syndrome, an autosomal recessive disorder of renal electrolyte transport.

REFERENCES

1. Hebert, S.C. 1995. An ATP-regulated, inwardly rectifying potassium channel from rat kidney (ROMK). Kidney Int. 48: 1010-1016.
2. Boim, M.A., et al. 1995. ROMK inwardly rectifying ATP-sensitive K^+ channel. II. Cloning and distribution of alternative forms. Am. J. Physiol. 268: F1132-F1140.
3. Kondo, C., et al. 1996. Cloning and functional expression of a novel isoform of ROMK inwardly rectifying ATP-dependent K^+ channel, ROMK6 (Kir1.1f). FEBS Lett. 399: 122-126.
4. Zolotnitskaya, A., et al. 1999. Developmental expression of ROMK in rat kidney. Am. J. Physiol. 276: F825-F836.
5. Flagg, T.P., et al. 1999. A mutation linked with Bartter's syndrome locks Kir 1.1a (ROMK1) channels in a closed state. J. Gen. Physiol. 114: 685-700.
6. Liou, H.H., et al. 1999. Regulation of ROMK1 channel by protein kinase A via a phosphatidylinositol 4,5-bisphosphate-dependent mechanism. Proc. Natl. Acad. Sci. USA 96: 5820-5825.
7. Loussouarn, G., et al. 2000. Structure and dynamics of the pore of inwardly rectifying K(ATP) channels. J. Biol. Chem. 275: 1137-1144.

CHROMOSOMAL LOCATION

Genetic locus: KCNJ1 (human) mapping to 11q24.3.

PRODUCT

ROM-K 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 ROM-K shRNA Plasmid (h): sc-42632-SH and ROM-K shRNA (h) Lentiviral Particles: sc-42632-V as alternate gene silencing products.

For independent verification of ROM-K (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-42632A, sc-42632B and sc-42632C.

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

ROM-K siRNA (h) is recommended for the inhibition of ROM-K 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

ROM-K (D-3): sc-393189 is recommended as a control antibody for monitoring of ROM-K 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 ROM-K gene expression knockdown using RT-PCR Primer: ROM-K (h)-PR: sc-42632-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.