SANTA CRUZ BIOTECHNOLOGY, INC.

RhBG siRNA (h): sc-88606

BACKGROUND

RhBG (Rh family, B glycoprotein), also known as ammonium transporter Rh type B or SLC42A2, is a 441 amino acid multi-pass membrane protein and ammonium transporter belonging to the ammonium transporter (TC 2.A.49) family. As one of two non-erythroid members of the Rhesus (Rh) protein subfamily, RhBG exists as five alternatively spliced isoforms and is expressed at highest levels in kidney where it localizes to the epithelial linings of convoluted tubules and loops of Henle; lower levels are found in liver and ovary. RhBG interacts with Ankyrin G and requires a *cis*-tyrosine-based signal to anchor itself to the basolateral cell membrane of kidney epithelial cells where it facilitates ammonium transport. Subject to post-translational N-terminal glycosylation, RhBG contains 12 transmembrane domains and is encoded by a gene mapping to human chromosome 1q22.

REFERENCES

1. Liu, Z., et al. 2001. Rh type B glycoprotein is a new member of the Rh superfamily and a putative ammonia transporter in mammals. J. Biol. Chem. 276: 1424-1433.
2. Ludewig, U. 2004. Electroneutral ammonium transport by basolateral Rhesus B glycoprotein. J. Physiol. 559: 751-759.
3. Khademi, S., et al. 2004. Mechanism of ammonia transport by Amt/MEP/Rh: structure of AmtB at 1.35 A. Science 305: 1587-1594.
4. Zidi-Yahiaoui, N., et al. 2005. Human Rhesus B and Rhesus C glycoproteins: properties of facilitated ammonium transport in recombinant kidney cells. Biochem. J. 391: 33-40.
5. Lopez, C., et al. 2005. The ammonium transporter RhBG: requirement of a tyrosine-based signal and ankyrin-G for basolateral targeting and membrane anchorage in polarized kidney epithelial cells. J. Biol. Chem. 280: 8221-8228.
6. Huang, C.H. and Peng, J. 2005. Evolutionary conservation and diversification of Rh family genes and proteins. Proc. Natl. Acad. Sci. USA 102: 15512-15517.
7. Sohet, F., et al. 2008. Phosphorylation and ankyrin-G binding of the C-terminal domain regulate targeting and function of the ammonium transporter RhBG. J. Biol. Chem. 283: 26557-26567.

CHROMOSOMAL LOCATION

Genetic locus: RHBG (human) mapping to 1q22.

PRODUCT

RhBG 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 RhBG shRNA Plasmid (h): sc-88606-SH and RhBG shRNA (h) Lentiviral Particles: sc-88606-V as alternate gene silencing products.

For independent verification of RhBG (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-88606A, sc-88606B and sc-88606C.

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

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

RhBG (B-9): sc-398816 is recommended as a control antibody for monitoring of RhBG 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 RhBG gene expression knockdown using RT-PCR Primer: RhBG (h)-PR: sc-88606-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.