SANTA CRUZ BIOTECHNOLOGY, INC.

megalin siRNA (m): sc-40104

BACKGROUND

Members of the LDL receptor gene family, including LDLR (low density lipoprotein receptor), LRP (low density lipoprotein related protein), megalin (also designated GP330), VLDLR (very low density lipoprotein receptor) and ApoER2, are characterized by a cluster of cysteine-rich class A repeats, epidermal growth factor (EGF)-like repeats, YWTD repeats and an O-linked sugar domain. Megalin is expressed on the apical membrane domain of epithelial cells, including proximal kidney tubules, intestine and ependymal cells. Proper folding and trafficking of megalin is facilitated by the receptor-associated protein (RAP), a molecular chaperone that can block the uptake of all known ligands for megalin. Specifically, megalin mediates the uptake of apolipoprotein J (apoJ, also designated Clusterin), which is a binding protein for the β-Amyloid peptide, a peptide implicated in Alzheimer's disease. Megalin is also an antigenic determinant for Heymann nephritis in rats and may be important in the reabsorption of several molecules, including vitamin B_{12}, in the kidney.

REFERENCES

1. Kounnas, M.Z., et al. 1995. Identification of glycoprotein 330 as an endocytic receptor for apolipoprotein J/clusterin. J. Biol. Chem. 270: 13070-13075.
2. Hammad, S.M., et al. 1997. Interaction of apolipoprotein J-β-Amyloid-peptide complex with low density lipoprotein receptor-related protein-2/megalin. A mechanism to prevent pathological accumulation of β-Amyloid-peptide. J. Biol. Chem. 272: 18644-18649.
3. Gliemann, J. 1998. Receptors of the low density lipoprotein (LDL) receptor family in man. Multiple functions of the large family members via interaction with complex ligands. Biol. Chem. 379: 951-964.
4. Trommsdorff, M., et al. 1999. Reeler/disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. Cell 97: 689-701.
5. Mikhailenko, I., et al. 1999. Functional domains of the very low density lipoprotein receptor: molecular analysis of ligand binding and acid-dependent ligand dissociation mechanisms. J. Cell Sci. 112: 3269-3281.
6. Bu, G., et al. 2000. Role of rap in the biogenesis of lipoprotein receptors. Trends Cardiovasc. Med. 10: 148-155.

CHROMOSOMAL LOCATION

Genetic locus: Lrp2 (mouse) mapping to 2 C2.

PRODUCT

megalin siRNA (m) 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 megalin shRNA Plasmid (m): sc-40104-SH and megalin shRNA (m) Lentiviral Particles: sc-40104-V as alternate gene silencing products.

For independent verification of megalin (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-40104A, sc-40104B and sc-40104C.

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

megalin siRNA (m) is recommended for the inhibition of megalin expression in mouse 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

megalin (G-9): sc-515750 is recommended as a control antibody for monitoring of megalin 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 megalin gene expression knockdown using RT-PCR Primer: megalin (m)-PR: sc-40104-PR (20 µl, 496 bp). 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.