



SMIT siRNA (m): sc-44517

BACKGROUND

Myo-inositol is involved in many important aspects of cellular regulation including membrane structure, signal transduction and osmoregulation. It is taken up into cells by the sodium/myo-inositol cotransporter (SMIT). SMIT activity maintains intracellular concentrations of myo-inositol; it is upregulated in response to hypertonic stress. The human SMIT protein is encoded by the SLC5A3 gene, which maps to chromosome 21q22.11. It is expressed in many human tissues, such as brain, kidney and placenta. Specifically, SMIT is abundantly expressed throughout the whole brain and spinal cord in fetal rat, but is downregulated in adult rat brain with the exception of the choroid plexus, where SMIT expression remains high. In kidney, SMIT localizes to the basolateral membranes of the thick ascending limb of Henle (TAL) and the inner medullary collecting duct (IMCD). Impaired SMIT activity is implicated in the pathogenesis of diabetes and Down syndrome.

REFERENCES

1. Berry, G.T., et al. 1995. The human osmoregulatory Na⁺/myo-inositol cotransporter gene (SLC5A3): molecular cloning and localization to chromosome 21. *Genomics* 25: 507-513.
2. Wiese, T.J., et al. 1996. Localization and regulation of renal Na⁺/myo-inositol cotransporter in diabetic rats. *Kidney Int.* 50: 1202-1211.
3. Mallee, J.J., et al. 1997. The structural organization of the human Na⁺/myo-inositol cotransporter (SLC5A3) gene and characterization of the promoter. *Genomics* 46: 459-465.
4. Guo, W., et al. 1997. Developmental regulation of Na⁺/myo-inositol cotransporter gene expression. *Brain Res. Mol. Brain Res.* 51: 91-96.
5. Porcellati, F., et al. 1998. Human Na⁺/myo-inositol cotransporter gene: alternate splicing generates diverse transcripts. *Am. J. Physiol.* 274: C1215-C1225.
6. Yamauchi, A., et al. 1998. Expression of the Na⁺/myo-inositol cotransporter in the juxtaglomerular region. *Kidney Int. Suppl.* 67: S183-S185.

CHROMOSOMAL LOCATION

Genetic locus: Slc5a3 (mouse) mapping to 16 C4.

PRODUCT

SMIT 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SMIT shRNA Plasmid (m): sc-44517-SH and SMIT shRNA (m) Lentiviral Particles: sc-44517-V as alternate gene silencing products.

For independent verification of SMIT (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-44517A, sc-44517B and sc-44517C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

SMIT siRNA (m) is recommended for the inhibition of SMIT 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 μ M in 66 μ l. 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

SMIT (3A6): sc-293330 is recommended as a control antibody for monitoring of SMIT 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 SMIT gene expression knockdown using RT-PCR Primer: SMIT (m)-PR: sc-44517-PR (20 μ l). 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.