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SMIT shRNA (h) Lentiviral Particles: sc-44516-V

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

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- Wiese, T.J., et al. 1996. Localization and regulation of renal Na⁺/myo-inositol cotransporter in diabetic rats. *Kidney Int.* 50: 1202-1211.
- Guo, W., et al. 1997. Developmental regulation of Na⁺ / myo-inositol cotransporter gene expression. *Brain Res. Mol. Brain Res.* 51: 91-96.
- 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.
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CHROMOSOMAL LOCATION

Genetic locus: SLC5A3 (human) mapping to 21q22.11.

PRODUCT

SMIT shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see SMIT siRNA (h): sc-44516 and SMIT shRNA Plasmid (h): sc-44516-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

SMIT shRNA (h) Lentiviral Particles is recommended for the inhibition of SMIT expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

SMIT (C-20): sc-23142 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SMIT gene expression knockdown using RT-PCR Primer: SMIT (h)-PR: sc-44516-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.