SANTA CRUZ BIOTECHNOLOGY, INC.

PiT2 siRNA (h): sc-61361

BACKGROUND

The SLC20 family transport proteins were originally identified as retroviral receptors Glvr-1 and Ram-1, but are now designated sodium-dependent phosphate transporters 1 and 2 (PiT1 and PiT2). The PiT proteins function as sodium-phosphate cotransporters and are widely expressed, with high expression in bone, kidney and intestine. Both PiT1 and PiT2 are expressed on polarized epithelial cell membranes where they play a role in cellular phosphate homeostasis. PiT2 is a human receptor for amphotropic murine leukemia virus (A-MuLV). A-MuLV infects a variety of mammalian cell lines, including humans, making it a useful tool in gene transfer technology and as a vector for gene therapy. Retroviral vector systems are used in gene therapy that are designed to infect cells expressing PiT1 or PiT2.

REFERENCES

1. Sugai, J., et al. 2001. Identification of envelope determinants of feline leukemia virus subgroup B that permit infection and gene transfer to cells expressing human Pit1 or Pit2. J. Virol. 75: 6841-6849.
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3. Bottger, P., et al. 2002. Two highly conserved glutamate residues critical for type III sodium-depend phosphate transport revealed by uncoupling transport function from retroviral receptor function. J. Biol. Chem. 277: 42741-42747.
4. Bøttger, P., et al. 2004. The central half of Pit2 is not required for its function as a retroviral receptor. J. Virol. 78: 9564-9567.
5. Beer, C., et al. 2005. Caveola-dependent endocytic entry of amphotropic murine leukemia virus. J. Virol. 79: 10776-10787.
6. Homann, V., et al. 2005. Sodium-phosphate cotransporter in human salivary glands: molecular evidence for the involvement of NPT2b in acinar phosphate secretion and ductal phosphate reabsorption. Arch. Oral. Biol. 50: 759-768.
7. Sudo, T., et al. 2005. Hepatic regeneration promotes engraftment of intraportally transplanted islet cells. Surgery 137: 612-619.
8. Leung, J.C., et al. 2005. Expression of the rat renal PiT-2 phosphate transporter. Horm. Metab. Res. 37: 265-269.

CHROMOSOMAL LOCATION

Genetic locus: SLC20A2 (human) mapping to 8p11.21.

PRODUCT

PiT2 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 PiT2 shRNA Plasmid (h): sc-61361-SH and PiT2 shRNA (h) Lentiviral Particles: sc-61361-V as alternate gene silencing products.

For independent verification of PiT2 (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-61361A, sc-61361B and sc-61361C.

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

PiT2 siRNA (h) is recommended for the inhibition of PiT2 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

PiT2 (B-4): sc-377326 is recommended as a control antibody for monitoring of PiT2 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 PiT2 gene expression knockdown using RT-PCR Primer: PiT2 (h)-PR: sc-61361-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.