SANTA CRUZ BIOTECHNOLOGY, INC.

THTR1 siRNA (h): sc-106612

BACKGROUND

Humans lack biosynthesis pathways for the micronutrients thiamine and folate; however, regulation of these vitamins is necessary for normal cellular function. The SLC19A gene family products mediate membrane transport of these molecules across the membrane to meet cellular requirements; in particular, two transporter proteins differentially import and export thiamine. In the liver as well as other tissues, THTR1 is responsible for the cellular accumulation, that is the import, of thiamine. Uptake depends on many factors, including sodium levels, pH, saturation of thiamine, presence of structural analogues such as oxythiamin and amprolium, as well as membrane transport inhibitors like amiloride. The gene encoding THTR1, SLC19A2, is regulated by GKLF, NF-1 and SP-1. Mutations of the SLC19A2 gene cause thiamine deficiency disorders such as thiamine-responsive megaloblastic anemia (TRMA) by interfering with either the functionality or intracellular targeting of THTR1.

REFERENCES

1. Boulware, M.J., et al. 2003. Polarized expression of members of the solute carrier SLC192 gene family of water-soluble multivitamin transporters: implications for physiological function. Biochem. J. 376: 43-48.
2. Subramanian, V.S., et al. 2003. Cell biology of the human thiamine transporter-1 (hTHTR1). Intracellular trafficking and membrane targetting mechanisms. J. Biol. Chem. 278: 3976-3984.
3. Said, H.M., et al. 2003. Cellular and molecular aspects of thiamin uptake by human liver cells: studies with cultured Hep G2 cells. Biochim. Biophys. Acta 1567: 106-112.
4. Reidling, J.C., et al. 2003. *In vitro* and *in vivo* characterization of the minimal promoter region of human thiamin transporter SLC19A2. Am. J. Physiol., Cell Physiol. 285: 633-641.
5. Baron, D., et al. 2003. Disruption of transport activity in a D93H mutant thiamine transporter 1, from a Rogers syndrome family. Eur. J. Biochem. 270: 4469-4477.

CHROMOSOMAL LOCATION

Genetic locus: SLC19A2 (human) mapping to 1q24.2.

PRODUCT

THTR1 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 THTR1 shRNA Plasmid (h): sc-106612-SH and THTR1 shRNA (h) Lentiviral Particles: sc-106612-V as alternate gene silencing products.

For independent verification of THTR1 (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-106612A, sc-106612B and sc-106612C.

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 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

THTR1 siRNA (h) is recommended for the inhibition of THTR1 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

THTR1 (R-5): sc-100649 is recommended as a control antibody for monitoring of THTR1 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 THTR1 gene expression knockdown using RT-PCR Primer: THTR1 (h)-PR: sc-106612-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.