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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic in



Klotho shRNA (h) Lentiviral Particles: sc-43883-V



The Power to Question

BACKGROUND

In Greek mythology the fate known as Klotho is a goddess who spins the thread of life. In mice, a deficiency in Klotho (KI) gene expression leads to various systemic phenotypes resembling human aging. Characteristics of the Klotho-deficient mouse include arteriosclerosis, osteoporosis, ectopic calcification and skin atrophy together with growth retardation, short lifespan and infertility. Mice deficient in Klotho show barely detectable amounts of white adipose tissue yet their brown adipose tissue (BAT) is comparably the same as in a normal genotype, suggesting that Klotho influences adipose differentiation. Mouse and human Klotho gene products are both characteristic type I transmembrane proteins that are approximately 80% homologous. The amino-terminal extracellular domain has two internal repeats, known as KL-1 and KL-2, which have partial sequence homology to β-glucosidases and lactase glycosylceramidase, suggesting a role for Klotho in sphingolipid metabolism. The human Klotho gene maps to chromosome 13q13.1 and encodes a 1,012 amino acid protein that is abundant in the kidney and brain. Chronic renal failure (CRF) patients express lower levels of Klotho mRNA and protein in the kidneys.

REFERENCES

- 1. Mori, K., et al. 2000. Disruption of Klotho gene causes an abnormal energy homeostasis in mice. Biochem. Biophys. Res. Commun. 278: 665-670.
- Mizuno, I., et al. 2001. Upregulation of the Klotho gene expression by thyroid hormone and during adipose differentiation in 3T3-L1 adipocytes. Life Sci. 68: 2917-2923.
- Koh, N., et al. 2001. Severely reduced production of Klotho in human chronic renal failure kidney. Biochem. Biophys. Res. Commun. 280: 1015-1020.
- Fukino, K., et al. 2002. Regulation of angiogenesis by the aging suppressor gene Klotho. Biochem. Biophys. Res. Commun. 293: 332-337.

CHROMOSOMAL LOCATION

Genetic locus: KL (human) mapping to 13q13.1.

PRODUCT

Klotho 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 μ l frozen stock containing 1.0 x 10 6 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 Klotho siRNA (h): sc-43883 and Klotho shRNA Plasmid (h): sc-43883-SH as alternate gene silencing products.

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.

APPLICATIONS

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 10 6 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

Klotho (E-21): sc-22220 is recommended as a control antibody for monitoring of Klotho 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 Klotho gene expression knockdown using RT-PCR Primer: Klotho (h)-PR: sc-43883-PR (20 μ I, 506 bp). 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.

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.

Santa Cruz Biotechnology, Inc. 1.800.457.3801 831.457.3801 **Europe** +00800 4573 8000 49 6221 4503 0 **www.scbt.com**