SANTA CRUZ BIOTECHNOLOGY, INC.

Klotho siRNA (m): sc-77344

BACKGROUND

In Greek mythology the fate known as Klotho is a goddess who spins the thread of life. In mice, a deficiency in Klotho (Kl) 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 life-span 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 13q12 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.
2. 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.
3. Koh, N., et al. 2001. Severely reduced production of Klotho in human chronic renal failure kidney. Biochem. Biophys. Res. Commun. 280: 1015-1020.
4. 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 (mouse) mapping to 5 G3.

PRODUCT

Klotho 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Klotho shRNA Plasmid (m): sc-77344-SH and Klotho shRNA (m) Lentiviral Particles: sc-77344-V as alternate gene silencing products.

For independent verification of Klotho (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-77344A, sc-77344B and sc-77344C.

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.

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

Klotho siRNA (m) is recommended for the inhibition of Klotho 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 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

Klotho (A-9): sc-515942 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 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 Klotho gene expression knockdown using RT-PCR Primer: Klotho (m)-PR: sc-77344-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.