

# Cystinosin siRNA (h): sc-105264

## BACKGROUND

Cystinosis is an autosomal recessive disorder resulting from defective lysosomal transport of cystine and present at birth as a failure to thrive, rickets and proximal renal tubular acidosis. The human CTNS gene on chromosome 17p13.2 encodes the protein Cystinosin, and mutations in CTNS are responsible for nephropathic cystinosis. The CTNS promoter contains an Sp1 binding element. Cystinosin is an integral membrane protein containing seven transmembrane domains that functions as a H<sup>+</sup>-driven transporter responsible for cystine export from lysosomes. In humans, Cystinosin is expressed abundantly in pancreas, kidney (mature and fetal) and skeletal muscle. The mouse homolog to CTNS encodes a protein which is expressed in all tissues except skeletal muscle. In the cell, Cystinosin co-localizes with LAMP-2 to lysosomes. A C-terminal GYDQL sorting motif within Cystinosin is critical for lysosomal localization.

## REFERENCES

1. Town, M., et al. 1998. A novel gene encoding an integral membrane protein is mutated in nephropathic cystinosis. *Nat. Genet.* 18: 319-324.
2. Cherqui, S., et al. 2000. Identification and characterization of the murine homologue of the gene responsible for cystinosis, Ctns. *BMC Genomics* 1: 2.
3. Pornphutkul, C., et al. 2001. The promoter of a lysosomal membrane transporter gene, CTNS, binds Sp-1, shares sequences with the promoter of an adjacent gene, CARKL, and causes cystinosis if mutated in a critical region. *Am. J. Hum. Genet.* 69: 712-721.
4. Kalatzis, V., et al. 2001. Cystinosin, the protein defective in cystinosis, is a H<sup>+</sup>-driven lysosomal cystine transporter. *EMBO J.* 20: 5940-5949.
5. Cherqui, S., et al. 2001. The targeting of Cystinosin to the lysosomal membrane requires a tyrosine-based signal and a novel sorting motif. *J. Biol. Chem.* 276: 13314-13321.

## CHROMOSOMAL LOCATION

Genetic locus: CTNS (human) mapping to 17p13.2.

## PRODUCT

Cystinosin 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Cystinosin shRNA Plasmid (h): sc-105264-SH and Cystinosin shRNA (h) Lentiviral Particles: sc-105264-V as alternate gene silencing products.

For independent verification of Cystinosin (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-105264A, sc-105264B and sc-105264C.

## PROTOCOLS

See our web site at [www.scbt.com](http://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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

Cystinosin siRNA (h) is recommended for the inhibition of Cystinosin 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  $\mu$ M in 66  $\mu$ l. 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

Cystinosin (F-12): sc-100703 is recommended as a control antibody for monitoring of Cystinosin 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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 Cystinosin gene expression knockdown using RT-PCR Primer: Cystinosin (h)-PR: sc-105264-PR (20  $\mu$ l, 434 bp). 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.