

# CTH siRNA (r): sc-270000

## BACKGROUND

CTH (cystathionine  $\gamma$ -lyase), also known as CSE or  $\gamma$ -cystathionase, is a member of the *trans*-sulfuration enzyme family and participates in the *trans*-sulfuration pathway. CTH is a cytoplasmic enzyme produced in the cytosol and is responsible for catalyzing the pyridoxal phosphate-dependent  $\beta$ -disulfide elimination reaction resulting in ammonium, pyruvate and thiocysteine. The thiocysteine that is produced may then react with other thiols (or cysteine) and form hydrogen sulfide ( $H_2S$ ). Thus, CTH is the major  $H_2S$ -producing enzyme in kidney, liver, vascular smooth muscle cells and enterocytes. The endogenous production of  $H_2S$  plays a significant role in the regulation of cellular functions, including cell growth, hyperpolarization of cell membranes, modulation of neuronal excitability and relaxation of smooth muscle cells. Mutations in the gene encoding CTH can result in the autosomal recessive disease cystathioninuria; a disorder characterized by the unusual accumulation of plasma cystathionine causing increased urinary excretion.

## REFERENCES

1. Lu, Y., et al. 1992. Cloning and nucleotide sequence of human liver cDNA encoding for cystathionine  $\gamma$ -lyase. *Biochem. Biophys. Res. Commun.* 189: 749-758.
2. Yang, G., et al. 2004. Cystathionine  $\gamma$ -lyase overexpression inhibits cell proliferation via a  $H_2S$ -dependent modulation of ERK 1/2 phosphorylation and p21<sup>Cip/WAK-1</sup>. *J. Biol. Chem.* 279: 49199-49205.
3. Dominy, J.E. and Stipanuk, M.H. 2004. New roles for cysteine and transsulfuration enzymes: production of  $H_2S$ , a neuromodulator and smooth muscle relaxant. *Nutr. Rev.* 62: 348-353.
4. Ishii, I., et al. 2004. Murine cystathionine  $\gamma$ -lyase: complete cDNA and genomic sequences, promoter activity, tissue distribution and developmental expression. *Biochem. J.* 381: 113-123.
5. Kamoun, P. 2004. Endogenous production of hydrogen sulfide in mammals. *Amino Acids* 26: 243-254.
6. Kamoun, P. 2004.  $H_2S$ , a new neuromodulator. *Med. Sci.* 20: 697-700.
7. Schicho, R., et al. 2006. Hydrogen sulfide is a novel prosecretory neuro-modulator in the Guinea-pig and human colon. *Gastroenterology* 131: 1542-1552.

## CHROMOSOMAL LOCATION

Genetic locus: Cth (rat) mapping to 2q45.

## PRODUCT

CTH siRNA (r) 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 CTH shRNA Plasmid (r): sc-270000-SH and CTH shRNA (r) Lentiviral Particles: sc-270000-V as alternate gene silencing products.

For independent verification of CTH (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270000A, sc-270000B and sc-270000C.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at  $-20^\circ C$  with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at  $-20^\circ C$ , 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

CTH siRNA (r) is recommended for the inhibition of CTH expression in rat 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

CTH (F-1): sc-374249 is recommended as a control antibody for monitoring of CTH 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<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> 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<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CTH gene expression knockdown using RT-PCR Primer: CTH (r)-PR: sc-270000-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ C$  and the extension temperature should be  $68-72^\circ C$ .

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.