

CPS1 siRNA (h): sc-35099

BACKGROUND

The multicompound protein, carbamoyl-phosphate synthetase-aspartate carbamoyl transferase-dihydro-ototase (CAD), consists of three distinct proteins, carbamoyl phosphate synthetase 2 (CPS2), aspartate transcarbamylase, and dihydro-ototase, which catalyze the second and third steps of pyrimidine biosynthesis. CAD is allosterically regulated by the phosphorylation of CPS2 by cyclic AMP-dependent protein kinase, and this activation enables CPS2 to catalyze the rate-limiting step of pyrimidine synthesis. CAD is expressed in brain and skeletal muscle. A related protein, carbamoyl phosphate synthetase 1 (CPS1) is expressed in liver. CPS1 catalyzes the rate-limiting step in the urea cycle, and deficiency of CPS1 is an autosomal recessive disorder that causes hyperammonemia.

REFERENCES

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- Haraguchi, Y., et al. 1991. Cloning and sequence of a cDNA encoding human carbamyl-phosphate synthetase I: molecular analysis of hyperammonemia. *Gene* 107: 335-340.
- Schofield, J.P., et al. 1999. Mice deficient in the urea-cycle enzyme, carbamoyl-phosphate synthetase I, die during the early neonatal period from hyperammonemia. *Hepatology* 29: 181-185.
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CHROMOSOMAL LOCATION

Genetic locus: CPS1 (human) mapping to 2q34.

PRODUCT

CPS1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CPS1 shRNA Plasmid (h): sc-35099-SH and CPS1 shRNA (h) Lentiviral Particles: sc-35099-V as alternate gene silencing products.

For independent verification of CPS1 (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-35099A, sc-35099B and sc-35099C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CPS1 siRNA (h) is recommended for the inhibition of CPS1 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 μ M in 66 μ 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

CPS1 (B-1): sc-376190 is recommended as a control antibody for monitoring of CPS1 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 CPS1 gene expression knockdown using RT-PCR Primer: CPS1 (h)-PR: sc-35099-PR (20 μ l). 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.