SANTA CRUZ BIOTECHNOLOGY, INC.

CPS2 siRNA (h): sc-41457

BACKGROUND

The multicomplex protein, carbamoyl-phosphate synthetase-aspartate carbamoyl transferase-dihydro-orotase (CAD), consists of three distinct proteins, carbamoyl phosphate synthetase 2 (CPS2), aspartate transcarbamylase, and dihydro-orotase, 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

1. Otsuki, T., et al. 1981. Phosphorylation and dephosphorylation of carbamoyl-phosphate synthetase II complex of rat ascites hepatoma cells. J. Biochem. 89: 1367-1374.
2. Carrey, E.A., et al. 1985. Phosphorylation and activation of hamster carbamyl-phosphate synthetase II by cAMP-dependent protein kinase. A novel mechanism for regulation of pyrimidine nucleotide biosynthesis. EMBO J. 4: 3735-3742.
3. Cammer, W., et al. 1991. Localization of the multifunctional protein CAD in astrocytes of rodent brain. J. Histochem. Cytochem. 39: 695-700.
4. 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.
5. 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.
6. Hewagama, A., et al. 1999. Functional linkage between the glutaminase and synthetase domains of carbamoyl-phosphate synthetase. Role of Serine 44 in carbamoyl-phosphate synthetase-aspartate carbamoyl transferase-dihydro-orotase (CAD). J. Biol. Chem. 274: 28240-28245.
7. Graves, L.M., et al. 2000. Regulation of carbamoyl-phosphate synthetase by MAP kinase. Nature 403: 328-332.

CHROMOSOMAL LOCATION

Genetic locus: CAD (human) mapping to 2p23.3.

PRODUCT

CPS2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CPS2 shRNA Plasmid (h): sc-41457-SH and CPS2 shRNA (h) Lentiviral Particles: sc-41457-V as alternate gene silencing products.

For independent verification of CPS2 (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-41457A, sc-41457B and sc-41457C.

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

CPS2 siRNA (h) is recommended for the inhibition of CPS2 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 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

CPS2 (F-6): sc-376072 is recommended as a control antibody for monitoring of CPS2 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 CPS2 gene expression knockdown using RT-PCR Primer: CPS2 (h)-PR: sc-41457-PR (20 µl, 550 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.

PROTOCOLS

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