SANTA CRUZ BIOTECHNOLOGY, INC.

CTPS1 siRNA (h): sc-78858

BACKGROUND

CTPS (cytidine-5'-triphosphate synthase) is a 591 amino acid protein that contains one glutamine amidotransferase type-1 domain and is involved in pyrimidine metabolism. CTPS catalyzes the ATP-dependent conversion of UTP to CTP, a rate-limiting reaction that requires either ammonia or L-glutamine as a nitrogen source. Via its catalytic activity, CTPS plays an important role in the synthesis of nucleic acids and is crucial for proper cell growth and development. The function of CTPS is regulated by a variety of mechanisms, including phosphorylation by protein kinase C (PKC), an event that can either stimulate or inhibit CTPS activity. The gene encoding CTPS is located in a region on chromosome 1 that is often associated with the progression of several tumor types, suggesting a possible role for CTPS in tumorigenesis.

REFERENCES

1. Whelan, J., et al. 1993. Clustered base substitutions in CTP synthetase conferring drug resistance in Chinese hamster ovary cells. Nat. Genet. 3: 317-322.
2. Verschuur, A.C., et al. 1999. Cytidine triphosphate synthase activity and mRNA expression in normal human blood cells. Biol. Chem. 380: 41-46.
3. Goto, M., et al. 2004. Crystal structures of CTP synthetase reveal ATP, UTP, and glutamine binding sites. Structure 12: 1413-1423.
4. Endrizzi, J.A., et al. 2005. Mechanisms of product feedback regulation and drug resistance in cytidine triphosphate synthetases from the structure of a CTP-inhibited complex. Biochemistry 44: 13491-13499.
5. Han, G.S., et al. 2005. Expression of human CTP synthetase in *Saccharomyces cerevisiae* reveals phosphorylation by protein kinase A. J. Biol. Chem. 280: 38328-38336.
6. Kursula, P., et al. 2006. Structure of the synthetase domain of human CTP synthetase, a target for anticancer therapy. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 62: 613-617.
7. Chang, Y.F., et al. 2007. Phosphorylation of human CTP synthetase 1 by protein kinase C: identification of Ser(462) and Thr(455) as major sites of phosphorylation. J. Biol. Chem. 282: 17613-17622.
8. Higgins, M.J., et al. 2007. Regulation of human cytidine triphosphate synthetase 1 by glycogen synthase kinase 3. J. Biol. Chem. 282: 29493-29503.

CHROMOSOMAL LOCATION

Genetic locus: CTPS1 (human) mapping to 1p34.2.

PRODUCT

CTPS1 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 CTPS1 shRNA Plasmid (h): sc-78858-SH and CTPS1 shRNA (h) Lentiviral Particles: sc-78858-V as alternate gene silencing products.

For independent verification of CTPS1 (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-78858A, sc-78858B and sc-78858C.

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

CTPS1 siRNA (h) is recommended for the inhibition of CTPS1 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

CTPS1 (2G7-1D10): sc-293266 is recommended as a control antibody for monitoring of CTPS1 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 CTPS1 gene expression knockdown using RT-PCR Primer: CTPS1 (h)-PR: sc-78858-PR (20 µl). 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.