SANTA CRUZ BIOTECHNOLOGY, INC.

OTC siRNA (h): sc-91306

BACKGROUND

OTC (ornithine carbamoyltransferase), also known as OTCase, is a 354 amino acid protein that belongs to the ATCase/OTCase family of proteins. Expressed in liver and intestinal mucosa, OTC localizes to the mitochondrial matrix and exists as a homotrimer. Specifically, OTC plays a vital role in the urea cycle, catalyzing the second step in this pathway: the formation of L-citrulline from L-orthinine and carbamoyl phosphate. In humans, the urea cycle is an important pathway to detoxification of ammonia. Mutations in the gene encoding OTC are associated with the X-linked disorder OTCD (ornithine carbamoyltransferase deficiency). OTCD is a disorder of the urea cycle characterized by hyperammonemia. In males, OTCD is fatal, whereas females express variable symptoms. In addition, the OTC gene localizes near a region of the X chromosome that is associated with Duchenne muscular dystrophy, suggesting a possible role in that disease as well.

REFERENCES

1. Lindgren, V., de Martinville, B., Horwich, A.L., Rosenberg, L.E. and Francke, U. 1984. Human ornithine transcarbamylase locus mapped to band Xp21.1 near the Duchenne muscular dystrophy locus. Science 226: 698-700.
2. Hata, A., Tsuzuki, T., Shimada, K., Takiguchi, M., Mori, M. and Matsuda, I. 1986. Isolation and characterization of the human ornithine transcarbamylase gene: structure of the 5'-end region. J. Biochem. 100: 717-725.
3. Tuchman, M., Holzknecht, R.A., Gueron, A.B., Berry, S.A. and Tsai, M.Y. 1992. Six new mutations in the ornithine transcarbamylase gene detected by single-strand conformational polymorphism. Pediatr. Res. 32: 600-604.
4. Trivedi, M., Zafar, S., Spalding, M.J. and Jonnalagadda, S. 2001. Ornithine transcarbamylase deficiency unmasked because of gastrointestinal bleeding. J. Clin. Gastroenterol. 32: 340-343.
5. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 300461. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: OTC (human) mapping to Xp11.4.

PRODUCT

OTC 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 OTC shRNA Plasmid (h): sc-91306-SH and OTC shRNA (h) Lentiviral Particles: sc-91306-V as alternate gene silencing products.

For independent verification of OTC (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-91306A, sc-91306B and sc-91306C.

PROTOCOLS

See our web site at 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 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

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

OTC (E-9): sc-515791 is recommended as a control antibody for monitoring of OTC 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 OTC gene expression knockdown using RT-PCR Primer: OTC (h)-PR: sc-91306-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.