SANTA CRUZ BIOTECHNOLOGY, INC.

UROD siRNA (h): sc-88548

BACKGROUND

Uroporphyrinogen decarboxylase, also known as UROD or UPD, is a 367 amino acid protein that exists as a homodimer. UROD is the fifth enzyme in the human heme biosynthetic pathway and is responsible for the conversion of uroporphyrinogen to coproporphyrinogen through the removal of four carboxymethyl side chains. Mutations in the UROD gene are responsible for three autosomal disorders in humans: familial porphyria cutanea tarda (f-PCT), sporadic porphyria cutanea tarda (s-PCT) and hepatoerythropoietic porphyria (HEP). F-PCT is an autosomal dominant disorder characterized by late-onset light-sensitive dermititis. High levels of uroporphyrin excretion in the urine and varying degrees of liver damage are associated with this disease. S-PCT is an idiosyncratic form of PCT that is characterized by a reduction of liver enzymes. HEP is an autosomal recessive disorder that affects infants. It is characterized by excessive excretion of acetate-substituted porphyrins and accumulation of protoporphyrin in erythrocytes.

REFERENCES

1. Moran-Jimenez, M.J., et al. 1996. Uroporphyrinogen decarboxylase: complete human gene sequence and molecular study of three families with hepatoerythropoietic porphyria. Am. J. Hum. Genet. 58: 712-721.
2. Phillips, J.D., et al. 1997. Characterization and crystallization of human uroporphyrinogen decarboxylase. Protein Sci. 6: 1343-1346.
3. Akhtar, R.A., et al. 1998. Chromosomal linkage analysis of porphyria in mice induced by hexachlorobenzene-iron synergism: a model of sporadic porphyria cutanea tarda. Pharmacogenetics 8: 485-494.
4. Christiansen, L., et al. 1999. Screening for mutations in the uroporphyrinogen decarboxylase gene using denaturing gradient gel electrophoresis. Identification and characterization of six novel mutations associated with familial PCT. Hum. Mutat. 14: 222-232.
5. Phillips, J.D., et al. 2001. Functional consequences of naturally occurring mutations in human uroporphyrinogen decarboxylase. Blood 98: 3179-3185.
6. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 176100. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: UROD (human) mapping to 1p34.1.

PRODUCT

UROD 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 UROD shRNA Plasmid (h): sc-88548-SH and UROD shRNA (h) Lentiviral Particles: sc-88548-V as alternate gene silencing products.

For independent verification of UROD (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-88548A, sc-88548B and sc-88548C.

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

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

UROD (C-4): sc-365297 is recommended as a control antibody for monitoring of UROD 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 UROD gene expression knockdown using RT-PCR Primer: UROD (h)-PR: sc-88548-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.