

UROS siRNA (m): sc-61761

BACKGROUND

Uroporphyrinogen-III synthase (UROS), also designated hydroxymethylbilane hydrolyase, is a 265-amino acid ubiquitously expressed protein that is predominantly found in skeletal muscle, heart muscle, the caudate nucleus, and the amygdala. UROS is the fourth enzyme in the human heme biosynthetic pathway and is responsible for the conversion of hydroxymethylbilane to the cyclic tetrapyrrole, uroporphyrinogen III. UROS is a monomer involved in porphyrin biosynthesis by the Shemin pathway. Defects in the UROS gene can cause molecular lesions that lead to the autosomal recessive Gunther disease, also known as congenital erythropoietic porphyria (CEP). CEP is characterized by accumulation of uroporphyrin I in the bone marrow, peripheral blood, and other organs, often causing death early in adult life.

REFERENCES

1. Tsai, S.F., et al. 1987. Purification and properties of uroporphyrinogen III synthase from human erythrocytes. *J. Biol. Chem.* 262: 1268-1273.
2. Tsai, S.F., et al. 1988. Human uroporphyrinogen III synthase: molecular cloning, nucleotide sequence, and expression of a full-length cDNA. *Proc. Natl. Acad. Sci. USA* 85: 7049-7053.
3. Astrin, K.H., et al. 1991. Regional assignment of the to chromosome 10q25.2→q26.3. *Hum. Genet.* 87: 18-22.
4. Xu, W., et al. 1995. Uroporphyrinogen-III synthase: molecular cloning, nucleotide sequence, expression of a mouse full-length cDNA, and its localization on mouse chromosome 7. *Genomics* 26: 556-562.
5. Geronimi, F., et al. 2003. Lentivirus-mediated gene transfer of uroporphyrinogen III synthase fully corrects the porphyric phenotype in human cells. *J. Mol. Med.* 81: 310-320.
6. Torres, G., et al. 2003. HLA-DQA1 and polymarker in the UROS population from Peruvian Highland. *J. Forensic Sci.* 48: 1426-1427.
7. Omata, Y., et al. 2004. Purification and characterization of human uroporphyrinogen III synthase expressed in *Escherichia coli*. *J. Biochem.* 136: 211-220.
8. de Villiers, J.N., et al. 2005. Overrepresentation of the founder PPOX gene mutation R59W in a South African patient with severe clinical manifestation of porphyria. *Exp. Dermatol.* 14: 50-55.

CHROMOSOMAL LOCATION

Genetic locus: Uros (mouse) mapping to 7 F3.

PRODUCT

UROS siRNA (m) 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 UROS shRNA Plasmid (m): sc-61761-SH and UROS shRNA (m) Lentiviral Particles: sc-61761-V as alternate gene silencing products.

For independent verification of UROS (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-61761A, sc-61761B and sc-61761C.

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

UROS siRNA (m) is recommended for the inhibition of UROS expression in mouse 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

UROS (A-8): sc-365877 is recommended as a control antibody for monitoring of UROS 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 UROS gene expression knockdown using RT-PCR Primer: UROS (m)-PR: sc-61761-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.