

# CYP27B1 siRNA (h): sc-60479

## BACKGROUND

The cytochrome P450 proteins are monooxygenases that catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. P450 enzymes are classified into subfamilies based on their sequence similarities. CYP27B1, a 508-amino acid protein that belongs to the XXVIIIB subfamily of the cytochrome P450 family, localizes to the mitochondrion and is expressed in the kidney. The CYP27B1 protein catalyzes the conversion of 25-hydroxyvitamin D<sub>3</sub> (25(OH)D) to 1- $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D) and functions in calcium metabolism, normal bone growth, and tissue differentiation. Mutations in the gene which encodes for CYP27B1 cause vitamin D-dependent rickets type 1 (VDDR-1), also designated pseudo-vitamin D deficiency rickets (PDDR), an autosomal recessive disease characterized by early onset of rickets with hypocalcemia and muscle weakness.

## REFERENCES

1. Dardenne, O., et al. 2001. Targeted inactivation of the 25-hydroxyvitamin D<sub>3</sub>-1 $\alpha$ -hydroxylase gene (CYP27B1) creates an animal model of pseudovitamin D-deficiency rickets. *Endocrinology* 142: 3135-3141.
2. Sawada, N., et al. 2001. Structure-function analysis of CYP27B1 and CYP27A1. Studies on mutants from patients with vitamin D-dependent rickets type I (VDDR-I) and cerebrotendinous xanthomatosis (CTX). *Eur. J. Biochem.* 268: 6607-6615.
3. Dardenne, O., et al. 2003. Correction of the abnormal mineral ion homeostasis with a high-calcium, high-phosphorus, high-lactose diet rescues the PDDR phenotype of mice deficient for the 25-hydroxyvitamin D-1 $\alpha$ -hydroxylase (CYP27B1). *Bone* 32: 332-340.

## CHROMOSOMAL LOCATION

Genetic locus: CYP27B1 (human) mapping to 12q14.1.

## PRODUCT

CYP27B1 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CYP27B1 shRNA Plasmid (h): sc-60479-SH and CYP27B1 shRNA (h) Lentiviral Particles: sc-60479-V as alternate gene silencing products.

For independent verification of CYP27B1 (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-60479A, sc-60479B and sc-60479C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

CYP27B1 siRNA (h) is recommended for the inhibition of CYP27B1 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  $\mu$ M in 66  $\mu$ 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

CYP27B1 (G-5): sc-515903 is recommended as a control antibody for monitoring of CYP27B1 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CYP27B1 gene expression knockdown using RT-PCR Primer: CYP27B1 (h)-PR: sc-60479-PR (20  $\mu$ l, 410 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Slominski, A.T., et al. 2011. 20-hydroxyvitamin D<sub>2</sub> is a noncalcemic analog of vitamin D with potent antiproliferative and prodifferentiation activities in normal and malignant cells. *Am. J. Physiol., Cell Physiol.* 300: C526-C541.
2. Geng, S., et al. 2011. Effects of 25-hydroxyvitamin D<sub>3</sub> on proliferation and osteoblast differentiation of human marrow stromal cells require CYP27B1/1 $\alpha$  hydroxylase. *J. Bone Miner. Res.* 26: 1145-1153.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.