



CYP21A2 siRNA (h): sc-60478

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. CYP21A2 localizes to the endoplasmic reticulum and hydroxylates steroids at the 21 position. Activity of CYP21A2 is required for the synthesis of steroid hormones, including cortisol and aldosterone. Mutations in this gene is the primary cause of congenital adrenal hyperplasia (CAH), an autosomal recessive disorder. Gene conversion events involving the functional CYP21A2 gene (C21B) and a related pseudogene (C21A) located near the C21B gene may account for the majority of cases of steroid 21-hydroxylase deficiency.

REFERENCES

1. Chang, S.F. and Chung, B.C. 1995. Difference in transcriptional activity of two homologous CYP21A genes. *Mol. Endocrinol.* 9: 1330-1336.
2. Nelson, D.R., et al. 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 6: 1-42.
3. Peterson, J.A., et al. 1997. P450BM-3; a tale of two domains—or is it three? *Steroids* 62: 117-123.
4. Araujo, R.S., et al. 2005. Substitutions in the CYP21A2 promoter explain the simple-virilizing form of deficiency in patients harbouring a P30L mutation. *Clin. Endocrinol.* 62: 132-136.
5. Keen-Kim, D., et al. 2005. Validation and clinical application of a locus-specific polymerase chain reaction- and minisequencing-based assay for congenital adrenal hyperplasia (21 hydroxylase deficiency). *J. Mol. Diagn.* 7: 236-246.
6. Krone, N., et al. 2005. The residue E351 is essential for the activity of human 21-hydroxylase: evidence from a naturally occurring novel point mutation compared with artificial mutants generated by single amino acid substitutions. *J. Mol. Med.* 83: 561-568.

CHROMOSOMAL LOCATION

Genetic locus: CYP21A2 (human) mapping to 6p21.33.

PRODUCT

CYP21A2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CYP21A2 shRNA Plasmid (h): sc-60478-SH and CYP21A2 shRNA (h) Lentiviral Particles: sc-60478-V as alternate gene silencing products.

For independent verification of CYP21A2 (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-60478A, sc-60478B and sc-60478C.

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

CYP21A2 siRNA (h) is recommended for the inhibition of CYP21A2 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 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

CYP21A2 (B-7): sc-518265 is recommended as a control antibody for monitoring of CYP21A2 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) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) 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 CYP21A2 gene expression knockdown using RT-PCR Primer: CYP21A2 (h)-PR: sc-60478-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.