

Produktinformation



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Diagnostik & molekulare Diagnostik



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CYP17A1 siRNA (h): sc-45641



The Power to Question

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, such as CYP1A, CYP2A, CYP2C, CYP2D, CYP4A14, CYP7A, CYP7B, CYP8B, CYP11A, CYP17A1, CYP19 and CYP27A, based on sequence similarities. CYP17A (17 α -hydroxylase/17,20-lyase) is important for the conversion of pregnenolone and progesterone to dehydroepiandrosterone (DHEA) and androstenedione. In this process, it catalyzes both the 17- α -hydroxylation and the 17,20-lyase reaction. CYP17A1 is crucial during sexual development, both during fetal development and during puberty, and is intracellularly regulated by cAMP levels. Defects in the CYP17A1 gene, which encodes for the protein, may cause adrenal hyperplasia type V (AH-V) which is characterized by hypokalemia and hypertension. Male patients affected by AH-V do not undergo normal sexual differentiation and develop female external genitalia and do not undergo pubertal develoment.

REFERENCES

- 1. Ahlgren, R., et al. 1992. Compound heterozygous mutations (Arg 239—stop, Pro 342—Thr) in the CYP17 (P45017 α) gene lead to ambiguous external genitalia in a male patient with partial combined 17 α -hydroxy-lase/17,20-lyase deficiency. J. Clin. Endocrinol. Metab. 74: 667-672.
- 2. Yanase, T., et al. 1992. Molecular basis of apparent isolated 17,20-lyase deficiency: compound heterozygous mutations in the C-terminal region (Arg(496)—Cys, Gln(461)—Stop) actually cause combined 17 a-hydroxy-lase/17,20-lyase. Biochim. Biophys. Acta 1139: 275-279.
- 3. Monno, S., et al. 1993. Mutation of histidine 373 to leucine in cytochrome P450c17 causes 17 α -hydroxylase deficiency. J. Biol. Chem. 268: 25811-25817.
- 4. Fardella, C.E., et al. 1994. Point mutation of Arg440 to His in cytochrome P450c17 causes severe 17 α -hydroxylase deficiency. J. Clin. Endocrinol. Metab. 79: 160-164.

CHROMOSOMAL LOCATION

Genetic locus: CYP17A1 (human) mapping to 10q24.32.

PRODUCT

CYP17A1 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 CYP17A1 shRNA Plasmid (h): sc-45641-SH and CYP17A1 shRNA (h) Lentiviral Particles: sc-45641-V as alternate gene silencing products.

For independent verification of CYP17A1 (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-45641A, sc-45641B and sc-45641C.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

CYP17A1 siRNA (h) is recommended for the inhibition of CYP17A1 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 60 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

CYP17A1 (G-4): sc-376711 is recommended as a control antibody for monitoring of CYP17A1 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluo-rescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CYP17A1 gene expression knockdown using RT-PCR Primer: CYP17A1 (h)-PR: sc-45641-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.

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