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CYP17A1 shRNA (m) Lentiviral Particles: sc-45642-V

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 development.

REFERENCES

- 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 -hydroxylase/17,20-lyase deficiency. *J. Clin. Endocrinol. Metab.* 74: 667-672.
- 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 α -hydroxylase/17,20-lyase. *Biochim. Biophys. Acta* 1139: 275-279.
- 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.
- Fardella, CE., 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 (mouse) mapping to 19 C3.

PRODUCT

CYP17A1 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see CYP17A1 siRNA (m): sc-45642 and CYP17A1 shRNA Plasmid (m): sc-45642-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

CYP17A1 shRNA (m) Lentiviral Particles is recommended for the inhibition of CYP17A1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

CYP17A1 (C-17): sc-46081 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (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 (m)-PR: sc-45642-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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