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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

CYP24 shRNA (m) Lentiviral Particles: sc-44653-V

BACKGROUND

P450 enzymes constitute a family of monooxygenase enzymes that are involved in the metabolism of a wide array of endogenous and xenobiotic compounds. P450 enzymes can be classified, based on their sequence similarities, into distinct subfamilies, which include CYP1A and CYP2A. The P450 family member, CYP19, catalyzes the conversion of C19 steroids to estrogens in various tissues, including placenta, gonads, adipose tissue, skin and brain. P450 cholesterol 7 α -hydroxylase, CYP7A1, is the rate limiting enzyme of bile acid synthesis in the liver, and its expression is mediated by the bile acid receptor FXR. CYP27A1 catalyzes vitamin D 25-hydroxylation and is localized to the mitochondria in kidney and liver. Overexpression of CYP24 (encoding vitamin D 24 hydroxylase) is likely to lead to abrogation of growth control mediated by vitamin D.

REFERENCES

1. Nelson, D.R., et al. 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 6: 1-42.
2. Peterson, J.A., et al. 1997. P450BM-3; a tale of two domains—or is it three? *Steroids* 62: 117-123.
3. Bulun, S.E., et al. 1997. Endocrine disorders associated with inappropriately high aromatase expression. *J. Steroid Biochem. Mol. Biol.* 61: 133-139.
4. Braunstein, G.D. 1999. Aromatase and gynecomastia. *Endocr. Relat. Cancer* 6: 315-324.
5. Albertson, D.G., et al. 2000. Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene. *Nat. Genet.* 25: 144-146.

CHROMOSOMAL LOCATION

Genetic locus: Cyp24a1 (mouse) mapping to 2 H3.

PRODUCT

CYP24 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 CYP24 siRNA (m): sc-44653 and CYP24 shRNA Plasmid (m): sc-44653-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.

PROTOCOLS

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

APPLICATIONS

CYP24 siRNA (m) is recommended for the inhibition of CYP24 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

CYP24 (H-87): sc-66851 is recommended as a control antibody for monitoring of CYP24 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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 goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CYP24 gene expression knockdown using RT-PCR Primer: CYP24 (m)-PR: sc-44653-PR (20 μ l, 598 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.