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CYP3A4 shRNA (h) Lentiviral Particles: sc-43711-V

BACKGROUND

Cytochrome P450 3A (CYP3A) genes encode monooxygenases—enzymes which catalyze drug metabolism and the synthesis of cholesterol, steroids and other lipids. CYP3A, the most abundant p450 enzyme in human liver, is responsible for the metabolism of more than 50% of all clinical drugs. CYP3A family members localize in organs that associate with drug disposition, including the liver, gastrointestinal tract and kidney. The CYP3A cluster maps to gene locus 7q21.3-q22.1 and consists of four genes (CYP3A4, CYP3A5, CYP3A7 and CYP3A43) and two pseudogenes (CYP3A5P1 and CYP3A5P2). CYP3A4 is abundant in the endoplasmic reticulum of liver cells and upper intestinal enterocytes. CYP3A4 expression is inducible by glucocorticoids pharmacological agents.

REFERENCES

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- Patel, J., et al. 2001. Strategies to overcome simultaneous P-glycoprotein-mediated efflux and CYP3A4-mediated metabolism of drugs. *Pharmacogenomics* 2: 401-415.
- Kapucuoglu, N., et al. 2003. Expression of CYP3A4 in human breast tumor and non-tumor tissues. *Cancer Lett.* 202: 17-23.
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CHROMOSOMAL LOCATION

Genetic locus: CYP3A4 (human) mapping to 7q22.1.

PRODUCT

CYP3A4 shRNA (h) 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 CYP3A4 siRNA (h): sc-43711 and CYP3A4 shRNA Plasmid (h): sc-43711-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

CYP3A4 shRNA (h) Lentiviral Particles is recommended for the inhibition of CYP3A4 expression in human 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

CYP3A4 (HL3): sc-53850 is recommended as a control antibody for monitoring of CYP3A4 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) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CYP3A4 gene expression knockdown using RT-PCR Primer: CYP3A4 (h)-PR: sc-43711-PR (20 μ l, 575 bp). 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.

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