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CYP1B1 shRNA (h) Lentiviral Particles: sc-44546-V

BACKGROUND

Cytochrome P450 1B1 (CYP1B1) is a key enzyme involved in the production of potentially carcinogenic estrogen metabolites and the activation of environmental carcinogens and is the predominant member of the CYP1 family expressed in normal breast tissue and breast cancer. Estrogen has been proposed to trigger breast cancer development via an initiating mechanism involving its metabolite, catechol estrogen (CE). CYP1B1 catalyzes the conversion of 17- β -estradiol to the catechol estrogen metabolites 2-OH-E2 and 4-OH-E2 which have both been postulated to be involved in mammary carcinogenesis. Genetic polymorphisms in CYP1B1 may play an important role in human prostate carcinogenesis as well. Polymorphism of the CYP1B1 gene at codon 432 (Val \rightarrow Leu) is associated with a change in catalytic function.

REFERENCES

1. Bailey L.R., et al. 1998. Association of cytochrome P450 1B1 (CYP1B1) polymorphism with steroid receptor status in breast cancer. *Cancer Res.* 58: 5038-5041.
2. Tang Y.M., et al. 2000. Human CYP1B1 Leu432Val gene polymorphism: ethnic distribution in African-Americans, Caucasians and Chinese; oestradiol hydroxylase activity; and distribution in prostate cancer cases and controls. *Pharmacogenetics* 10: 761-766.
3. Kocabas N.A., et al. 2002. Cytochrome P450 CYP1B1 and catechol O-methyltransferase (COMT) genetic polymorphisms and breast cancer susceptibility in a Turkish population. *Arch. Toxicol.* 11: 643-649.
4. Saintot M., et al. 2004. Interaction between genetic polymorphism of cytochrome P450 1B1 and environmental pollutants in breast cancer risk. *Eur. J. Cancer Prev.* 13: 83-86.

CHROMOSOMAL LOCATION

Genetic locus: CYP1B1 (human) mapping to 2p22.2.

PRODUCT

CYP1B1 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 CYP1B1 siRNA (h): sc-44546 and CYP1B1 shRNA Plasmid (h): sc-44546-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

CYP1B1 shRNA (h) Lentiviral Particles is recommended for the inhibition of CYP1B1 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

Lambda 5 (A-1): sc-398932 is recommended as a control antibody for monitoring of Lambda 5 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 CYP1B1 gene expression knockdown using RT-PCR Primer: CYP1B1 (h)-PR: sc-44546-PR (20 μ l, 559 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.