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UGT1A shRNA (h2) Lentiviral Particles: sc-44476-V

BACKGROUND

Glucuronidation, an important bile acid detoxification pathway, is catalyzed by enzymes belonging to the UDP-glucuronosyltransferase (UGT) superfamily. UGT genes are classified into the UGT1A and UGT2B subfamilies. Although each subfamily and each isoform shows tissue-specific patterns of distribution, the underlying mechanisms for this tissue specificity have not been fully elucidated. The human UDP-glucuronosyltransferase 1 (UGT1) locus encodes at least ten UGT1A proteins (UGT1A1-UGT1A10) that play a prominent role in drug and xenobiotic metabolism. Research indicates that nuclear receptors such as pregnane X receptor (PXR), constitutive androstane receptor (CAR) and peroxisome proliferator-activated receptor (PPAR) can regulate UGTs, which may contribute to the tissue-specific expression pattern of UGTs. Deficiency in the expression and/or activity of UGTs may lead to genetic and acquired diseases such as Crigler-Najjar syndrome and Gilbert syndrome. Based on their ability to catalyze the glucuronidation of xenobiotics and endobiotics, UGTs play a critical role in hormonal homeostasis, energy metabolism, bilirubin clearance and xenobiotic detoxification.

REFERENCES

1. Moghrabi, N., et al. 1992. Chromosomal assignment of human phenol and bilirubin UDP-glucuronosyltransferase genes (UGT1A-subfamily). *Ann. Hum. Genet.* 56: 81-91.
2. Owens, I.S., et al. 1996. The novel UGT1 gene complex links bilirubin, xenobiotics, and therapeutic drug metabolism by encoding UDP-glucuronosyltransferase isozymes with a common carboxyl terminus. *J. Pharmacokin. Biopharm.* 24: 491-508.
3. Ciotti, M., et al. 1997. Genetic defects at the UGT1 locus associated with Crigler-Najjar type I disease, including a prenatal diagnosis. *Am. J. Med. Genet.* 68: 173-178.
4. Strassburg, C.P., et al. 1997. Differential down-regulation of the UDP-glucuronosyltransferase 1A locus is an early event in human liver and biliary cancer. *Cancer Res.* 57: 2979-2985.

CHROMOSOMAL LOCATION

Genetic locus: UGT1A8 (human) mapping to 2q37.1.

PRODUCT

UGT1A shRNA (h2) 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 UGT1A siRNA (h2): sc-44476 and UGT1A shRNA Plasmid (h2): sc-44476-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

UGT1A shRNA (h2) Lentiviral Particles is recommended for the inhibition of UGT1A1 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

UGT1A (B-4): sc-271268 is recommended as a control antibody for monitoring of UGT1A1 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 UGT1A1 gene expression knockdown using RT-PCR Primer: UGT1A (h2)-PR: sc-44476-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.

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