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ATP7B shRNA (h) Lentiviral Particles: sc-44491-V

BACKGROUND

The copper efflux transporters ATP7A and ATP7B sequester intracellular copper into the vesicular secretory pathway for export from the cell. ATP7A functions as a transmembrane copper-translocating P-type ATPase and plays a vital role in systemic copper absorption in the gut and copper reabsorption in the kidney. Polarized epithelial cells such as Madin-Darby canine kidney cells are a physiologically relevant model for systemic copper absorption and reabsorption *in vivo*. Although ATP7A is not detectable in most normal tissues, it is expressed in a considerable fraction of many common tumor types. Increased expression of ATP7A renders cells resistant to Cisplatin and carboplatin. Mutations in the ATP7A gene result in Menkes disease, which is fatal in early childhood. Mutations in the ATP7B gene lead to the autosomal recessive disorder Wilson disease, characterized by neurological symptoms and hepatic damage.

REFERENCES

1. Samimi, G., et al. 2003. Increase in expression of the copper transporter ATP7A during platinum drug-based treatment is associated with poor survival in ovarian cancer patients. *Clin. Cancer Res.* 9: 5853-5859.
2. Samimi, G., et al. 2004. Modulation of the cellular pharmacology of Cisplatin and its analogs by the copper exporters ATP7A and ATP7B. *Mol. Pharmacol.* 66: 25-32.
3. Greenough, M., et al. 2004. Signals regulating trafficking of Menkes (MNK; ATP7A) copper-translocating P-type ATPase in polarized MDCK cells. *Am. J. Physiol., Cell Physiol.* 287: C1463-C1471.
4. Song, I.S., et al. 2004. Role of human copper transporter Ctr1 in the transport of platinum-based antitumor agents in Cisplatin-sensitive and Cisplatin-resistant cells. *Mol. Cancer Ther.* 3: 1543-1549.

CHROMOSOMAL LOCATION

Genetic locus: ATP7B (human) mapping to 13q14.3.

PRODUCT

ATP7B 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 ATP7B siRNA (h): sc-44491 and ATP7B shRNA Plasmid (h): sc-44491-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

ATP7B shRNA (h) Lentiviral Particles is recommended for the inhibition of ATP7B 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

ATP7B (A-11): sc-373964 is recommended as a control antibody for monitoring of ATP7B 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 ATP7B gene expression knockdown using RT-PCR Primer: ATP7B (h)-PR: sc-44491-PR (20 μ l, 593 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.