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Transcobalamin II shRNA (h) Lentiviral Particles: sc-45320-V

BACKGROUND

Transcobalamin I (TCI) and Transcobalamin II (TCII) are secreted proteins belonging to the eukaryotic cobalamin transport proteins family and also to the vitamin B12-binding protein family. The genes encoding these proteins map to chromosome 11q12.1 and 22q12.2, respectively. Transcobalamin I is a constituent of secondary granules in neutrophils, while Transcobalamin II binds cobalamin and mediates its transport into cells. These plasma proteins are expressed in various tissues and secretions.

REFERENCES

1. Kalra, S., et al. 2004. Cobalamin (vitamin B12) binding, phylogeny, and synteny of human transcobalamin. *Arch. Biochem. Biophys.* 431: 189-196.
2. Cheeramakara, C., et al. 2005. Elevation of serum transcobalamin II in patients with scrub typhus. *Southeast Asian J. Trop. Med. Public Health* 36: 113-117.
3. Chen, X., et al. 2005. Influence of cobalamin deficiency compared with that of cobalamin absorption on serum holo-transcobalamin II. *Am. J. Clin. Nutr.* 81: 110-114.
4. Fedosov, S.N., et al. 2005. Mapping the functional domains of human transcobalamin using monoclonal antibodies. *FEBS J.* 272: 3887-3898.
5. Swanson, D.A., et al. 2005. Evaluation of transcobalamin II polymorphisms as neural tube defect risk factors in an Irish population. *Birth Defects Res. A Clin. Mol. Teratol.* 73: 239-244.
6. Martinelli, M., et al. 2006. Study of four genes belonging to the folate pathway: transcobalamin 2 is involved in the onset of non-syndromic cleft lip with or without cleft palate. *Hum. Mutat.* 27: 294.

CHROMOSOMAL LOCATION

Genetic locus: TCN2 (human) mapping to 22q12.2.

PRODUCT

Transcobalamin II 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 Transcobalamin II siRNA (h): sc-45320 and Transcobalamin II shRNA Plasmid (h): sc-45320-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

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

Transcobalamin II (A-5): sc-137017 is recommended as a control antibody for monitoring of Transcobalamin II 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 Transcobalamin II gene expression knockdown using RT-PCR Primer: Transcobalamin II (h)-PR: sc-45320-PR (20 μ l). 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.