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apoA-V shRNA (h) Lentiviral Particles: sc-44527-V

BACKGROUND

Apolipoproteins are protein components of plasma lipoproteins. The human apoA-I gene encodes a single chain, 243 amino acid protein which promotes cholesterol efflux from tissues to the liver for excretion. Apolipoprotein A-I is the major protein component of high density lipoprotein (HDL) in the plasma. It can function as a cofactor for lecithin cholesterolacyltransferase (LCAT), which is responsible for the formation of most plasma cholesteryl esters. The human apoA-II gene encodes the second most abundant protein of HDL particles, where it influences plasma levels of free fatty acids (FFA). The human apoA-IV gene encodes a 396 amino acid preprotein, which after proteolytic processing is secreted from the intestine in association with chylomicron particles. ApoA-IV is a potent activator of lecithin-cholesterol acyltransferase (LCAT) *in vitro*. The human apoA-V gene encodes a 366 amino acid protein that is believed to be an important determinant of plasma triglyceride levels.

REFERENCES

- Vergnes, L., et al. 1997. The apolipoprotein A-I/C-III/A-IV gene cluster: apoC-III and apoA-IV expression is regulated by two common enhancers. *Biochim. Biophys. Acta* 1348: 299-310.
- Qin, S., et al. 2000. Phospholipid transfer protein gene knock-out mice have low high density lipoprotein levels, due to hypercatabolism, and accumulate apoA-IV-rich lamellar lipoproteins. *J. Lipid. Res.* 41: 269-276.
- Fournier, N., et al. 2000. Human apoA-IV overexpression in transgenic mice induces cAMP-stimulated cholesterol efflux from J774 macrophages to whole serum. *Arterioscler. Thromb. Vasc. Biol.* 20: 1283-1292.
- Deeg, M.A., et al. 2001. GPI-specific phospholipase D associates with an apoA-I- and apoA-IV-containing complex. *J. Lipid. Res.* 42: 442-451.

CHROMOSOMAL LOCATION

Genetic locus: APOA5 (human) mapping to 11q23.

PRODUCT

apoA-V 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 apoA-V siRNA (h): sc-44527 and apoA-V shRNA Plasmid (h): sc-44527-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

apoA-V shRNA (h) Lentiviral Particles is recommended for the inhibition of apoA-V 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

apoA-V (A-12): sc-393722 is recommended as a control antibody for monitoring of apoA-V 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 apoA-V gene expression knockdown using RT-PCR Primer: apoA-V (h)-PR: sc-44527-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.