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apoA-V siRNA (h): sc-44527

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.

CHROMOSOMAL LOCATION

Genetic locus: APOA5 (human) mapping to 11q23.3.

PRODUCT

apoA-V siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see apoA-V shRNA Plasmid (h): sc-44527-SH and apoA-V shRNA (h) Lentiviral Particles: sc-44527-V as alternate gene silencing products.

For independent verification of apoA-V (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44527A, sc-44527B and sc-44527C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

apoA-V siRNA (h) is recommended for the inhibition of apoA-V expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

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° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.