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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

apoD shRNA (h) Lentiviral Particles: sc-45518-V

BACKGROUND

Lipids, such as phospholipids, triacylglycerols and cholesterol, are weakly soluble in aqueous solution and therefore are transported by circulation as components of lipoproteins. Lipoproteins are globular particles that consist of a non-polar core of triacylglycerols and cholesteryl esters surrounded by phospholipid, cholesterol and an amphiphilic coating of protein, known as apolipoproteins (apo). These complexes allow the dissolution and shuttling of their non-polar lipid components. At least nine different apolipoproteins are distributed in significant amounts in different human lipoproteins. Apolipoprotein D (apoD) is a member of the lipocalin superfamily of transporter proteins that bind small hydrophobic molecules, including arachidonic acid (AA). The ability of apoD to bind AA implicates it in pathways associated with membrane phospholipid signal transduction and metabolism. apoD expression has been shown to correlate both with cell cycle arrest and with prognosis in several types of malignancy, including central nervous system astrocytomas and medulloblastomas.

REFERENCES

1. Yao, J.K., et al. 2005. Association of plasma apolipoproteins D with RBC membrane arachidonic acid levels in schizophrenia. *Schizophr. Res.* 72: 259-266.
2. Ganfornina, M.D., et al. 2005. Molecular characterization and developmental expression pattern of the chicken apolipoprotein D gene: implications for the evolution of vertebrate lipocalins. *Dev. Dyn.* 232: 191-199.
3. Hildebrand, M.S., et al. 2005. Expression of the carrier protein apolipoprotein D in the mouse inner ear. *Hear. Res.* 200: 102-114.
4. Utsunomiya, T., et al. 2005. Clinicopathologic and prognostic values of apolipoprotein D alterations in hepatocellular carcinoma. *Int. J. Cancer* 116: 105-109.
5. Desai, P.P., et al. 2005. Apolipoprotein D is a component of compact but not diffuse amyloid- β plaques in Alzheimer's disease temporal cortex. *Neurobiol. Dis.* 20: 574-582.

CHROMOSOMAL LOCATION

Genetic locus: APOD (human) mapping to 3q29.

PRODUCT

apoD 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 apoD siRNA (h): sc-45518 and apoD shRNA Plasmid (h): sc-45518-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

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

apoD (C-1): sc-373965 is recommended as a control antibody for monitoring of apoD 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 apoD gene expression knockdown using RT-PCR Primer: apoD (h)-PR: sc-45518-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.

PROTOCOLS

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