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ABCA7 shRNA (h) Lentiviral Particles: sc-45431-V

BACKGROUND

ATP-binding cassette (ABC) transporters are an evolutionarily conserved family of widely-expressed proteins that use ATP hydrolysis to catalyze the transport of various molecules across extracellular and intracellular membranes. Eukaryotic ABC transporters are largely responsible for trafficking hydrophobic compounds either within the cell as part of a metabolic process, outside the cell for transport to other organs, or for secretion from the body. The cholesterol-responsive transporter, ABCA7, maps to human chromosome 19 and mouse chromosome 10 and has been reported as a candidate regulator of ceramide transport in epidermal lipid reorganization. High expression levels of ABCA7 have been reported in myelolymphatic tissues, reticuloendothelial cells, peripheral leukocytes, thymus, spleen and bone marrow. This expression pattern of the two alternatively-spliced isoforms also indicates an involvement in lipid homeostasis in cells of the immune system, though the complete role of ABCA7 is not yet known. Full-length type I ABCA7 has shown plasma membrane localization, while the type II splicing variant has shown expression predominantly in the endoplasmic reticulum.

REFERENCES

1. Kaminski, W.E., et al. 2000. Genomic organization of the human cholesterol-responsive ABC transporter ABCA7: tandem linkage with the minor histocompatibility antigen HA-1 gene. *Biochem. Biophys. Res. Commun.* 278: 782-789.
2. Broccardo, C., et al. 2001. Comparative analysis of the promoter structure and genomic organization of the human and mouse ABCA7 gene encoding a novel ABCA transporter. *Cytogenet. Cell Genet.* 92: 264-270.
3. Ikeda, Y., et al. 2003. Post-transcriptional regulation of human ABCA7 and its function for the apoA-I-dependent lipid release. *Biochem. Biophys. Res. Commun.* 311: 313-318.

CHROMOSOMAL LOCATION

Genetic locus: ABCA7 (human) mapping to 19p13.3.

PRODUCT

ABCA7 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 ABCA7 siRNA (h): sc-45431 and ABCA7 shRNA Plasmid (h): sc-45431-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

ABCA7 shRNA (h) Lentiviral Particles is recommended for the inhibition of ABCA7 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

ABCA7 (P-16): sc-34559 is recommended as a control antibody for monitoring of ABCA7 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ABCA7 gene expression knockdown using RT-PCR Primer: ABCA7 (h)-PR: sc-45431-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.