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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) 



ABCG4 shRNA (h) Lentiviral Particles: sc-44489-V

BACKGROUND

ATP-binding cassette (ABC) transporters are an evolutionarily conserved family of proteins that catalyze the transport of molecules across extra- and intracellular membranes through the energy of ATP hydrolysis. The ABC gene family comprises seven subfamilies, ABC1, MDR/TAP, MRP, ALD, OABP, GCN20 and White. ABC proteins are either full molecules, with two nucleotide-binding folds (NBFs) and two sets of transmembrane domains (TMs), or half molecules, with one NBF and one set of TM domains. ABCG4, as well as all other half transporters, forms either a hetero- or homodimer in order to mediate transport function. A 646 amino acid protein, ABCG4 is important in macrophage lipid homeostasis, with the highest expression detected in brain, thymus, spleen and heart. The gene encoding for the ABCG4 protein maps to chromosome 11q23.3, a locus which includes several other genes involved in cholesterol and lipid metabolism. This locus also includes the locus for primary hypolipoproteinemia.

REFERENCES

1. Annino, T., et al. 2001. Human and mouse orthologs of a new ATP-binding cassette gene, ABCG4. *Cytogenet. Cell Genet.* 94: 196-201.
2. Engel, T., et al. 2001. The human ABCG4 gene is regulated by oxysterols and retinoids in monocyte-derived macrophages. *Biochem. Biophys. Res. Commun.* 288: 483-488.
3. Yoshikawa, M., et al. 2002. Molecular and cytogenetic characterization of the mouse ATP-binding cassette transporter ABCG4. *Gene* 293: 67-75.
4. Nakagawa, R., et al. 2002. ABCG2 confers resistance to indolocarbazole compounds by ATP-dependent transport. *Biochem. Biophys. Res. Commun.* 299: 669-675.

CHROMOSOMAL LOCATION

Genetic locus: ABCG4 (human) mapping to 11q23.3.

PRODUCT

ABCG4 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 ABCG4 siRNA (h): sc-44489 and ABCG4 shRNA Plasmid (h): sc-44489-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

ABCG4 shRNA (h) Lentiviral Particles is recommended for the inhibition of ABCG4 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

ABCG4 (E-17): sc-34872 is recommended as a control antibody for monitoring of ABCG4 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 ABCG4 gene expression knockdown using RT-PCR Primer: ABCG4 (h)-PR: sc-44489-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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 USER 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.