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CAT-1 shRNA (h) Lentiviral Particles: sc-44923-V

BACKGROUND

The cationic amino acid transporter (CAT) family of proteins are part of a larger superfamily, the amino acid-polyamine-organocation (APC) superfamily. High-affinity cationic amino acid transporter-1 (CAT-1), also designated ecotropic retroviral leukemia receptor homolog, ATRC1 or REC1L, is an ubiquitously expressed integral membrane protein. In non-hepatic tissues, CAT-1 acts as a high-affinity, low capacity permease that is important in cationic amino acid transport. CAT-1 is also a potential ecotropic retroviral leukemia receptor. SLC7A1, the gene encoding for the CAT-1 protein, maps to chromosome 13q12.3.

REFERENCES

1. Yoshimoto, T., et al. 1991. Molecular cloning and characterization of a novel human gene homologous to the murine ecotropic retroviral receptor. *Virology* 185: 10-17.
2. Albritton, L.M., et al. 1992. The human cationic amino acid transporter (ATRC1): physical and genetic mapping to 13q12-q14. *Genomics* 12: 430-434.
3. Kamath, S.G., et al. 1999. Identification of three cationic amino acid transporters in placental trophoblast: cloning, expression, and characterization of hCAT-1. *J. Membr. Biol.* 171: 55-62.
4. Zani, B.G., et al. 2005. Transport of extracellular L-arginine via cationic amino acid transporter is required during *in vivo* endothelial nitric oxide production. *Am. J. Physiol. Heart Circ. Physiol.* 289: H1381-H1390.
5. Li, C., et al. 2005. Interaction of the endothelial nitric oxide synthase with the CAT-1 arginine transporter enhances NO release by a mechanism not involving arginine transport. *Biochem. J.* 386: 567-574.

CHROMOSOMAL LOCATION

Genetic locus: SLC7A1 (human) mapping to 13q12.3.

PRODUCT

CAT-1 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 CAT-1 siRNA (h): sc-44923 and CAT-1 shRNA Plasmid (h): sc-44923-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

CAT-1 shRNA (h) Lentiviral Particles is recommended for the inhibition of CAT-1 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

CAT-1 (C-12): sc-33087 is recommended as a control antibody for monitoring of CAT-1 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 CAT-1 gene expression knockdown using RT-PCR Primer: CAT-1 (h)-PR: sc-44923-PR (20 μ l, 446 bp). 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.