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CysLT₁ Receptor shRNA (h) Lentiviral Particles: sc-43712-V

BACKGROUND

Cysteinyl leukotriene (CysLTs) induce intracellular calcium mobilization through the binding of two distinct seven-transmembrane, G protein-coupled receptors, designated CysLT₁ and CysLT₂ Receptors, to induce potent bronchoconstriction. Airway smooth muscle and macrophages express both receptor types, and additionally monocytes and eosinophils express CysLT₁ Receptor, while cardiac Purkinje cells, adrenal medulla, peripheral blood leukocytes and brain also utilize CysLT₂ Receptor. The effects of the CysLT receptors can be blocked by antagonists, indicating a therapeutic mechanism for the treatment of asthma and allergies.

REFERENCES

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2. Lynch, K.R., et al. 1999. Characterization of the human cysteinyl leukotriene CysLT₁ receptor. *Nature* 399: 789-793.
3. Heise, C.E., et al. 2000. Characterization of the human cysteinyl leukotriene 2 receptor. *J. Biol. Chem.* 275: 30531-30536.
4. Sjostrom, M., et al. 2001. Human umbilical vein endothelial cells generate leukotriene C₄ via microsomal glutathione S-transferase type 2 and express the CysLT₁ receptor. *Eur. J. Biochem.* 268: 2578-2586.
5. Maekawa, A., et al. 2001. Identification in mice of two isoforms of the cysteinyl leukotriene 1 receptor that result from alternative splicing. *Proc. Natl. Acad. Sci. USA* 98: 2256-2261.
6. Leff, A.R. 2001. Regulation of leukotrienes in the management of asthma: biology and clinical therapy. *Annu. Rev. Med.* 52: 1-14.

CHROMOSOMAL LOCATION

Genetic locus: CYSLTR1 (human) mapping to Xq21.1.

PRODUCT

CysLT₁ Receptor 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 µl frozen stock containing 1.0 x 10⁶ 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 CysLT₁ Receptor siRNA (h): sc-43712 and CysLT₁ Receptor shRNA Plasmid (h): sc-43712-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

CysLT₁ Receptor shRNA (h) Lentiviral Particles is recommended for the inhibition of CysLT₁ Receptor expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

CysLT₁ Receptor (C-16): sc-31172 is recommended as a control antibody for monitoring of CysLT₁ Receptor 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 CysLT₁ Receptor gene expression knockdown using RT-PCR Primer: CysLT₁ Receptor (h)-PR: sc-43712-PR (20 µl, 501 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.