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LL-37 shRNA (h) Lentiviral Particles: sc-43697-V

BACKGROUND

Cathelicidins are a family of antimicrobial proteins found in the peroxidase-negative granules of neutrophils. Along with the family of proteins known as defensins, cathelicidins participate in the first line of defense by preventing local infection and systemic invasion of microbes. CAP-18 precursor (FALL-39 peptide antibiotic, cationic anti-microbial protein, CAMP, HSD26) is a cathelicidin anti-microbial protein that contains the antibacterial peptide LL-37 (amino acids 134-170). In contrast to the defensins, which are cysteine-rich peptides that fold in β -pleated sheets, LL-37 is a cysteine-free peptide that can adopt an amphipathic α -helical conformation. LL-37 binds to bacterial lipopolysaccharides (LPS) and is a potent chemotactic factor for recruiting mast cells to sites of inflammation. LL-37 is present in inflammatory skin diseases that include psoriasis, sub-acute lupus erythematosus, dermatitis and nickel contact hypersensitivity. It is not found in normal skin epidermis. The secreted protein is expressed primarily in bone marrow, testis and neutrophils. The mouse and rat ortholog, CRAMP (cathelin-related antimicrobial peptide), is also part of the cathelicidin family of host defense peptides. These include precursors of potent antimicrobial peptides that direct antimicrobial activity against various microbial pathogens and also activate mesenchymal cells during wound repair. CRAMP is expressed in testis, spleen, stomach and intestine.

REFERENCES

1. Popsueva, A.E., et al. 1996. A novel murine cathelin-like protein expressed in bone marrow. *FEBS Lett.* 391: 5-8.
2. Gallo, R.L., et al. 1997. Identification of CRAMP, a cathelin-related anti-microbial peptide expressed in the embryonic and adult mouse. *J. Biol. Chem.* 272: 13088-13093.

CHROMOSOMAL LOCATION

Genetic locus: CAMP (human) mapping to 3p21.31.

PRODUCT

LL-37 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 LL-37 siRNA (h): sc-43697 and LL-37 shRNA Plasmid (h): sc-43697-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

LL-37 shRNA (h) Lentiviral Particles is recommended for the inhibition of LL-37 proteolytic fragment of CAP-18 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

LL-37 (D-5): sc-166770 is recommended as a control antibody for monitoring of LL-37 proteolytic fragment of CAP-18 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 LL-37 proteolytic fragment of CAP-18 gene expression knockdown using RT-PCR Primer: LL-37 (h)-PR: sc-43697-PR (20 μ l, 454 bp). 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 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.