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LL-37 siRNA (h): sc-43697

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 antimicrobial peptide expressed in the embryonic and adult mouse. *J. Biol. Chem.* 272: 13088-13093.
3. Frohm, M., et al. 1997. The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J. Biol. Chem.* 272: 15258-15263.
4. Sorensen, O., et al. 2001. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood* 97: 3951-3959.
5. Murakami, M., et al. 2002. Cathelicidin antimicrobial peptides are expressed in salivary glands and saliva. *J. Dent. Res.* 81: 845-850.

CHROMOSOMAL LOCATION

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

PRODUCT

LL-37 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see LL-37 shRNA Plasmid (h): sc-43697-SH and LL-37 shRNA (h) Lentiviral Particles: sc-43697-V as alternate gene silencing products.

For independent verification of LL-37 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43697A, sc-43697B and sc-43697C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

LL-37 siRNA (h) is recommended for the inhibition of LL-37 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

LL-37 (D-5): sc-166770 is recommended as a control antibody for monitoring of LL-37 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor LL-37 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° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.