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RANTES shRNA (m) Lentiviral Particles: sc-45573-V

BACKGROUND

Structurally, C-C or β -chemokines are characterized by a set of conserved, adjacent cysteines. Members of this family include MCP-1, MCP-2, MCP-3, MIP-1 α , MIP-1 β , RANTES and I-309. RANTES (regulated upon activation, normal T cell expressed and secreted) is expressed by platelets, eosinophils, fibroblasts, macrophages, endothelial cells and T lymphocytes. Consistent with its belonging to the chemokine family, RANTES exhibits strong chemoattractant activity towards monocytes and NK cells. I-309 was initially identified as a factor present in γ/δ T lymphocytes. I-309 cDNA encodes a protein 73 amino acids in length with one potential N-linked glycosylation site. Unlike the other members of the C-C family, I-309 does not induce chemotaxis in natural killer (NK) cells.

REFERENCES

1. Miller, M.D., et al. 1989. A novel polypeptide secreted by activated human T lymphocytes. *J. Immunol.* 143: 2907-2916.
2. Wells, T.N., et al. 1996. Selectivity and antagonism of chemokine receptors. *J. Leukoc. Biol.* 59: 53-60.
3. Taub, D.D., et al. 1996. β -chemokines costimulate lymphocyte cytolysis, proliferation and lymphokine production. *J. Leukoc. Biol.* 59: 81-89.
4. Wang, J.H., et al. 1996. Expression of RANTES by human bronchial epithelial cells *in vitro* and *in vivo* and the effect of corticosteroids. *Am. J. Respir. Cell Mol. Biol.* 14: 27-35.
5. Ying, S., et al. 1996. Human eosinophils express messenger RNA encoding RANTES and store and release biologically active RANTES protein. *Eur. J. Immunol.* 26: 70-76.
6. Loetscher, P., et al. 1996. Activation of NK cells by C-C chemokines. Chemotaxis, Ca²⁺ mobilization and enzyme release. *J. Immunol.* 156: 322-327.

CHROMOSOMAL LOCATION

Genetic locus: Ccl5 (mouse) mapping to 11 C.

PRODUCT

RANTES shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each viral contains 200 μ l 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 RANTES siRNA (m): sc-45573 and RANTES shRNA Plasmid (m): sc-45573-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

RANTES shRNA (m) Lentiviral Particles is recommended for the inhibition of RANTES expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l 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

RANTES (A-4): sc-365826 is recommended as a control antibody for monitoring of RANTES 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 RANTES gene expression knockdown using RT-PCR Primer: RANTES (m)-PR: sc-45573-PR (20 μ l). 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.