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MCP-1 shRNA (h) Lentiviral Particles: sc-43913-V

BACKGROUND

Eotaxin and the monocyte chemotactic proteins, MCP-1-5, form a subfamily of the C-C (or β) chemokines, which are characterized by a set of conserved adjacent cysteines. MCPs are produced by a variety of cells, including T lymphocytes, subsequent to their activation with cytokines such as IL-1, TNF α and IFN- γ . *In vitro* studies have shown that the MCP isoforms exhibit their chemotactic effects on different subpopulations of lymphocytes. MCP-1 is a potent basophil activator but does not affect eosinophils. MCP-1 levels are increased during infection and inflammation, which are both characterized by leukocyte infiltration. Two MCP-1 receptors, which differ in their carboxy-termini, have been identified.

REFERENCES

1. Charo, I.F., et al. 1994. Molecular cloning and functional expression of two monocyte chemoattractant protein -1 receptors reveals alternative splicing of the carboxyl-terminal tails. *Proc. Natl. Acad. Sci. USA* 91: 2752-2756.
2. Taub, D.D., et al. 1995. Monocyte chemotactic protein-1 (MCP-1), -2 and -3 are chemotactic for human T lymphocytes. *J. Clin. Invest.* 95: 1370-1376.
3. Weber, M., et al. 1995. Monocyte chemotactic protein MCP-2 activates human basophil and eosinophil leukocytes similar to MCP-3. *J. Immunol.* 154: 4166-4172.
4. Combadiere, C., et al. 1995. Monocyte chemoattractant protein-3 is a functional ligand for C-C chemokine receptors 1 and 2B. *J. Biol. Chem.* 270: 29671-29675.
5. Proost, P., et al. 1996. Human monocyte chemotactic proteins-2 and -3: structural and functional comparison with MCP-1. *J. Leukoc. Biol.* 59: 67-74.

CHROMOSOMAL LOCATION

Genetic locus: CCL2 (human) mapping to 17q12.

PRODUCT

MCP-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 MCP-1 siRNA (h): sc-43913 and MCP-1 shRNA Plasmid (h): sc-43913-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

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

MCP-1 (C-17): sc-1304 is recommended as a control antibody for monitoring of MCP-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 MCP-1 gene expression knockdown using RT-PCR Primer: MCP-1 (h)-PR: sc-43913-PR (20 μ l, 474 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.