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IP-10 shRNA (h) Lentiviral Particles: sc-43866-V

BACKGROUND

Chemokines are members of a superfamily of inducible, secreted, pro-inflammatory cytokines. Members of the chemokine family exhibit 20% to 50% homology in their predicted amino acid sequences and are divided into four subfamilies: C-C, C-X-C, C and C-X3-C. In the C-X-C or α subfamily, the first two of four cysteine motifs are separated by another amino acid residue. In the second subfamily, designated C-C or β , the first cysteines are adjacent. C subfamily members, also designated γ chemokines, lack the first and third cysteine residues of the conserved motif. In the C-X3-C, or δ subfamily, members have three amino acids between the two cysteines. The C-X-C chemokine subfamily includes IL-8, GRO $\alpha/\beta/\gamma$ (and the murine homologs KC, MIP-2 α and MIP-2 β), platelet basic protein, ENA-78, GCP-2, PF4, IP-10 (and its murine homolog, CRG) and MIG.

REFERENCES

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2. Schall, T.J. 1991. Biology of the RANTES/SIS cytokine family. *Cytokine* 3: 165-183.
3. Miller, M.D., et al. 1992. Biology and biochemistry of the chemokines: a family of chemotactic and inflammatory cytokines. *Crit. Rev. Immunol.* 12: 17-46.
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5. Roth, S.J., et al. 1995. C-C chemokines, but not the C-X-C chemokines interleukin-8 and interferon- γ inducible protein-10, stimulate transendothelial chemotaxis of T lymphocytes. *Eur. J. Immunol.* 25: 3482-3488.

CHROMOSOMAL LOCATION

Genetic locus: CXCL10 (human) mapping to 4q21.1.

PRODUCT

IP-10 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 IP-10 siRNA (h): sc-43866 and IP-10 shRNA Plasmid (h): sc-43866-SH as alternate gene silencing products.

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.

APPLICATIONS

IP-10 shRNA (h) Lentiviral Particles is recommended for the inhibition of IP-10 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

IP-10 (1): sc-101500 is recommended as a control antibody for monitoring of IP-10 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 IP-10 gene expression knockdown using RT-PCR Primer: IP-10 (h)-PR: sc-43866-PR (20 μ l). 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.

STORAGE

Store lentiviral particles at -80 $^{\circ}$ C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4 $^{\circ}$ 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.