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CKR-2 shRNA (m) Lentiviral Particles: sc-44766-V

BACKGROUND

C-C or β chemokine family members are characterized by a pair of adjacent cysteine residues and serve as potent chemoattractants and activators of monocytes and T cells. C-C chemokine receptor family members include CKR-1, CKR-2A, CKR-2B, CKR-3, CKR-4, CKR-5, CKR-6, CKR-7, CKR-8, CKR-9, CKR-10 and the Duffy blood group antigen. Each of these receptors are G protein coupled, seven pass transmembrane domain proteins whose major physiological role is to function in the chemotaxis of T cells and phagocytic cells to areas of inflammation. However, this receptor family has also been shown to facilitate viral infection. CKR-2 (C-C chemokine receptor type 2) is a 374 amino acid multi-pass membrane protein that belongs to the C-C chemokine receptor family and is expressed as two isoforms, designated CKR-2A and CKR-2B. Both CKR-2 isoforms function as receptors for a variety of proteins, including MCP-1 and MCP-3, thereby influencing intracellular calcium levels and affecting signal transduction throughout the cell.

REFERENCES

- Schweickart, V.L., et al. 1994. Cloning of human and mouse EBI1, a lymphoid-specific G-protein-coupled receptor encoded on human chromosome 17q12-q21.2. *Genomics* 23: 643-650.
- Deng, H., et al. 1996. Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 381: 661-666.
- Dragic, T., et al. 1996. HIV-1 entry into CD4⁺ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* 381: 667-673.
- Feng, Y., et al. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 272: 872-877.
- Alkhatib, G., et al. 1996. C-C CKR5: a RANTES, MIP-1 α , MIP-1 β receptor as a fusion cofactor for macrophage tropic HIV-1. *Science* 272: 1955-1958.

CHROMOSOMAL LOCATION

Genetic locus: Ccr2 (mouse) mapping to 9 F4.

PRODUCT

CKR-2 shRNA (m) 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 CKR-2 siRNA (m): sc-44766 and CKR-2 shRNA Plasmid (m): sc-44766-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

CKR-2 shRNA (m) Lentiviral Particles is recommended for the inhibition of CKR-2 expression in mouse 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

CKR-2 (N-14): sc-31563 is recommended as a control antibody for monitoring of CKR-2 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 CKR-2 gene expression knockdown using RT-PCR Primer: CKR-2 (m)-PR: sc-44766-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.

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.