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fractalkine siRNA (h): sc-43771

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. In the subfamily designated C-C or β , the first two cysteines are adjacent. In the C-X-C or α subfamily, the first two of four cysteine residues are separated by a single amino acid. C subfamily members, also designated γ chemokines, lack the first and third cysteine residues of the conserved motif. Chemokines in these three subfamilies are small, secreted proteins. Fractalkine, also designated neurotactin, is the first characterized member of a fourth chemokine subfamily. Fractalkine contains a novel C-X3-C motif in which the first two cysteines are separated by three amino acid residues. Fractalkine mRNA has been detected in brain and heart and is upregulated in microglia and endothelial cells by inflammatory signals. The protein exists both as a membrane-bound form and as a chemotactic soluble form.

REFERENCES

1. Oppenheim, J.J., et al. 1991. Properties of the novel proinflammatory supergene "intercrine" cytokine family. *Annu. Rev. Immunol.* 9: 617-648.
2. 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.
3. Taub, D.D., et al. 1993. Review of the chemokine meeting of the Third International Symposium of Chemotactic Cytokines. *Cytokine* 5: 175-179.
4. Schall, T.J., et al. 1994. Chemokines, leukocyte trafficking, and inflammation. *Curr. Opin. Immunol.* 6: 865-873.

CHROMOSOMAL LOCATION

Genetic locus: CX3CL1 (human) mapping to 16q21.

PRODUCT

fractalkine 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 fractalkine shRNA Plasmid (h): sc-43771-SH and fractalkine shRNA (h) Lentiviral Particles: sc-43771-V as alternate gene silencing products.

For independent verification of fractalkine (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-43771A, sc-43771B and sc-43771C.

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

fractalkine siRNA (h) is recommended for the inhibition of fractalkine 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

fractalkine (A-9): sc-166200 is recommended as a control antibody for monitoring of fractalkine 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 fractalkine gene expression knockdown using RT-PCR Primer: fractalkine (h)-PR: sc-43771-PR (20 μ l, 575 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

SELECT PRODUCT CITATIONS

1. Lee, A.S., et al. 2009. Epigallocatechin-3-O-gallate decreases tumor necrosis factor- α -induced fractalkine expression in endothelial cells by suppressing NF κ B. *Cell. Physiol. Biochem.* 24: 503-510.

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.