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Factor B shRNA (h) Lentiviral Particles: sc-44510-V

BACKGROUND

The complement component proteins, C3, C4 and C5, are potent anaphylatoxins that are released during complement activation. Binding of these proteins to their respective G protein-coupled receptors, C3 α R, C1R and C5 α R, induces proinflammatory events, such as cellular degranulation, smooth muscle contraction, arachidonic acid metabolism, cytokine release, leukocyte activation and cellular chemotaxis. Complement Factor B, also designated Properdin Factor B or PBF2, is part of the alternate pathway of the complement system and is cleaved by Factor D into two fragments: B α and B β . B β combines with complement Factor 3 β to produce the C3 or C5 convertase and plays a role in the differentiation and proliferation of preactivated B lymphocytes, lysis of erythrocytes, stimulation of lymphocyte blastogenesis and rapid spreading of peripheral blood monocytes. B α is important in inhibiting the proliferation of preactivated B lymphocytes. Adipsin, also designated complement Factor D, is a serine protease that cleaves complement Factor B and may be involved in obesity. Factor H controls the function of the alternative complement pathway. FHR-1 (complement Factor H related protein 1) may play a role in lipid metabolism.

REFERENCES

1. Woods, D.E., et al. 1982. Isolation of cDNA clones for the human complement protein Factor B, a class III major histocompatibility complex gene product. *Proc. Natl. Acad. Sci. USA* 79: 5661-5665.
2. Campbell, R.D., et al. 1983. Molecular cloning and characterization of the gene coding for human complement protein Factor B. *Proc. Natl. Acad. Sci. USA* 80: 4464-4468.
3. Mole, J.E., et al. 1984. Complete primary structure for the zymogen of human complement Factor B. *J. Biol. Chem.* 259: 3407-3412.
4. Wu, L.C., et al. 1987. Cell-specific expression of the human complement protein Factor B gene: evidence for the role of two distinct 5'-flanking elements. *Cell* 48: 331-342.

CHROMOSOMAL LOCATION

Genetic locus: CFB (human) mapping to 6p21.33.

PRODUCT

Factor B 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 Factor B siRNA (h): sc-44510 and Factor B shRNA Plasmid (h): sc-44510-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.

APPLICATIONS

Factor B shRNA (h) Lentiviral Particles is recommended for the inhibition of Factor B 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

Factor B (F-7): sc-271636 is recommended as a control antibody for monitoring of Factor B 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 Factor B gene expression knockdown using RT-PCR Primer: Factor B (h)-PR: sc-44510-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.

PROTOCOLS

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