SANTA CRUZ BIOTECHNOLOGY, INC.

C1s siRNA (h): sc-60301

BACKGROUND

The complement component proteins, C1, C3, C4, and C5, are potent anaphylatoxins that are released during complement activation. Binding of these proteins to their respective G protein-coupled receptors induces proinflammatory events, such as cellular degranulation, smooth muscle contraction, arachidonic acid metabolism, cytokine release, leukocyte activation, and cellular chemotaxis. C1q, together with proenzymes C1r and C1s, yield C1, the first component of the classical pathway of the serum complement system. C1 consists of a calcium dependent trimolecular complex of C1r, C1s and C1q in a 2:2:1 ratio. Activated C1s is in the form of a disulfide-linked heterodimer consisting of a heavy chain and a light chain. Defects in the gene encoding for C1s can cause selective C1s deficiency, a disorder characterized by early onset of various autoimmune diseases.

REFERENCES

1. Tosi, M., et al. 1987. Complete cDNA sequence of human complement CIs and close physical linkage of the homologous genes CIs and CIr. Biochemistry 26: 8516-8524.
2. Matsumoto, M., et al. 1989. Probing a C4/C4b-binding site on the γ-domain. J. Immunol. 142: 2743-2750.
3. Nakagawa, K., et al. 1999. Complement C1s activation in degenerating articular cartilage of rheumatoid arthritis patients: immunohistochemical studies with an active form specific antibody. Ann. Rheum. Dis. 58: 175-781.
4. Gaboriaud, C., et al. 2000. Crystal structure of the catalytic domain of human complement C1s: a serine protease with a handle. EMBO J. 19: 1755-1765.
5. Dragon-Durey, M.A., et al. 2001. Molecular basis of a selective C1s deficiency associated with early onset multiple autoimmune diseases. J. Immunol. 166: 7612-7616.
6. Gregory, L.A., et al. 2003. X-ray structure of the Ca^{2+}-binding interaction domain of C1s. Insights into the assembly of the C1 complex of complement. J. Biol. Chem. 278: 32157-32164.
7. Glovsky, M.M., et al. 2004. Complement determinations in human disease. Ann. Allergy Asthma Immunol. 93: 513-522.

CHROMOSOMAL LOCATION

Genetic locus: C1S (human) mapping to 12p13.31.

PRODUCT

C1s 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see C1s shRNA Plasmid (h): sc-60301-SH and C1s shRNA (h) Lentiviral Particles: sc-60301-V as alternate gene silencing products.

For independent verification of C1s (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-60301A, sc-60301B and sc-60301C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

C1s siRNA (h) is recommended for the inhibition of C1s 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 µM in 66 µl. 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

C1s (D-6): sc-365273 is recommended as a control antibody for monitoring of C1s 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 C1s gene expression knockdown using RT-PCR Primer: C1s (h)-PR: sc-60301-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.