

C3aR siRNA (h): sc-42840

BACKGROUND

Complement C3 precursor contains complement C3 β chain, complement C3 α chain, C3a anaphylatoxin, complement C3b α chain, complement C3c fragment, complement C3dg fragment, complement C3g fragment, complement C3d fragment and complement C3f fragment. C3a, C4a, and C5a are potent anaphylatoxins that are released during complement activation, a system of ligand-surface protein interactions specific to cells of hematopoietic lineage that aids in the elimination of pathogens. C3a and C5a secretion correlates with pathophysiological phenotypes such as asthma and bacterial meningitis. Binding of these proteins to their respective G protein-coupled receptors (C3aR, C5aR), which are present on the surface of myeloid leukocytes, induces proinflammatory events such as cellular degranulation, smooth muscle contraction, arachidonic acid metabolism, cytokine release, leukocyte activation and cellular chemotaxis. C3aR is expressed in brain and activated B-lymphocytes whereas C5aR is prevalent on the surface of hepatocyte, lung, smooth muscle, and endothelial cells. Upon activation, C3aR and C5aR are susceptible to rapid GRK-mediated phosphorylation and clathrin-coated vesicle targeting. C5aR utilizes the Ras-Raf-ERK1/2 cascade and couples to G_i/G₁₆ proteins.

REFERENCES

1. de Bruijn, M.H., et al. 1985. Human complement component C3: cDNA coding sequence and derived primary structure. *Proc. Natl. Acad. Sci. USA* 82: 708-712.
2. Buhl, A.M., et al. 1995. Mitogen-activated protein kinase activation requires two signal inputs from the human anaphylatoxin C5a receptor. *J. Biol. Chem.* 270: 19828-19832.

CHROMOSOMAL LOCATION

Genetic locus: C3AR1 (human) mapping to 12p13.31.

PRODUCT

C3aR 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 C3aR shRNA Plasmid (h): sc-42840-SH and C3aR shRNA (h) Lentiviral Particles: sc-42840-V as alternate gene silencing products.

For independent verification of C3aR (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-42840A, sc-42840B and sc-42840C.

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

C3aR siRNA (h) is recommended for the inhibition of C3aR 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

C3aR (D-12): sc-133172 is recommended as a control antibody for monitoring of C3aR 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 C3aR gene expression knockdown using RT-PCR Primer: C3aR (h)-PR: sc-42840-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ghannam, A., et al. 2014. Human complement C3 deficiency: Th1 induction requires T cell-derived complement C3a and CD46 activation. *Mol. Immunol.* 58: 98-107.

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.