

C5 siRNA (h): sc-42848

BACKGROUND

C3 α , C4 α and C5 α 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. Complement C5 precursor contains C5 α anaphylatoxin. C3 α and C5 α secretion correlates with pathophysiological phenotypes such as asthma and bacterial meningitis. Binding of these proteins to their respective G protein-coupled receptors (C3 α R, C5 α R), 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. C5 α R utilizes the Ras-Raf-ERK1/2 cascade, couples to G γ /G β proteins, and is prevalent on the surface of hepatocyte, lung, smooth muscle and endothelial cells. Upon activation, C3 α R and C5 α R are susceptible to rapid GRK-mediated phosphorylation and Clathrin-coated vesicle targeting. The C5 precursor is first processed by the removal of four basic residues, forming two chains, α and β , linked by a disulfide bond. C5 convertase activates C5 by cleaving the α chain, releasing C5 α anaphylatoxin and generating C5 β .

REFERENCES

1. de Buijn, 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.
3. Stahel, P.F., et al. 1997. TNF- α -mediated expression of the receptor for anaphylatoxin C5a on neurons in experimental *Listeria* meningoencephalitis. *J. Immunol.* 159: 861-869.
4. Langkabel, P., et al. 1999. Ligand-induced phosphorylation of anaphylatoxin receptors C3aR and C5aR is mediated by G protein-coupled receptor kinases. *Eur. J. Immunol.* 29: 3035-3046.
5. Settmacher, B., et al. 1999. Modulation of C3a activity: internalization of the human C3a receptor and its inhibition by C5a. *J. Immunol.* 162: 7409-7416.
6. Humbles, A.A., et al. 2000. A role for the C3a anaphylatoxin receptor in the effector phase of asthma. *Nature* 406: 998-1001.

CHROMOSOMAL LOCATION

Genetic locus: C5 (human) mapping to 9q33.2.

PRODUCT

C5 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 C5 shRNA Plasmid (h): sc-42848-SH and C5 shRNA (h) Lentiviral Particles: sc-42848-V as alternate gene silencing products.

For independent verification of C5 (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-42848A, sc-42848B and sc-42848C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

C5 siRNA (h) is recommended for the inhibition of C5 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

C5 (561): sc-52636 is recommended as a control antibody for monitoring of C5 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor C5 gene expression knockdown using RT-PCR Primer: C5 (h)-PR: sc-42848-PR (20 μ l, 503 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ 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.