# SZABO SCANDIC 

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## BACKGROUND

The complement component proteins C3, C4 and C5 are potent anaphylatoxins that are released during complement activation, a system of ligand-surface protein interactions specific to cells of hematopoietic lineage. These proteins belong to the $\alpha$-2-Macroglobulin family, but retain distinctive features including an anaphylatoxin domain and a netrin (NTR) domain. The human C4 gene is polymorphic at two loci, C4A and C4B, mapping to chromosome 6p21.33. C4A expresses the Rodgers ( Rg ) blood group Ag, while C4B expresses the Chido (Ch) blood group Ag. C4 is expressed as a precursor that is cleaved into $\alpha, \beta$ and $\gamma$ chains, all of which are non-identical cleavage products. The $\alpha$ chain of C4 may be cleaved to produce an acidic isotype, C4a, which reacts with amino groups, and a basic isotype, C4b, which reacts with hydroxyl groups. Deficiency in the C4 gene is associated with autoimmune or immune complex disorders, such as systemic lupus erythematosus.

## REFERENCES

1. Hugli, T.E. 1984. Structure and function of the anaphylatoxins. Springer Semin. Immunopathol. 7: 193-219.
2. Yu, C.Y., et al. 1986. Structural basis of the polymorphism of human complement components C4A and C4B: gene size, reactivity and antigenicity. EMBO J. 5: 2873-2881.
3. Andoh, A., et al. 1997. Molecular characterization of complement components (C3, C4 and factor B) in human saliva. J. Clin. Immunol. 17: 404-407.
4. Martinez, O.P., et al. 2001. Genetics of human complement component C4 and evolution the central MHC. Front. Biosci. 6: D904-D913.
5. Blanchong, C.A., et al. 2001. Genetic, structural and functional diversities of human complement components C4A and C4B and their mouse homologs, SIp and C4. Int. Immunopharmacol. 1: 365-392.
6. Jaatinen, T., et al. 2002. Characterization of a de novo conversion in human complement C4 gene producing a C4B5-like protein. J. Immunol. 168: 5652-5658.

## CHROMOSOMAL LOCATION

Genetic locus: C4a/C4b (mouse) mapping to 17 B1.

## PRODUCT

C4 siRNA (m) is a pool of 3 target-specific $19-25 \mathrm{nt}$ siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a $10 \mu \mathrm{M}$ solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see C4 shRNA Plasmid (m): sc-42845-SH and C4 shRNA (m) Lentiviral Particles: sc-42845-V as alternate gene silencing products.
For independent verification of $\mathrm{C4}(\mathrm{~m})$ gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-42845A, sc-42845B and sc-42845C.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at $-20^{\circ} \mathrm{C}$ with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at $-20^{\circ} \mathrm{C}$, avoid contact with RNAses and repeated freeze thaw cycles.
Resuspend lyophilized siRNA duplex in $330 \mu \mathrm{l}$ of the RNAse-free water provided. Resuspension of the siRNA duplex in $330 \mu \mathrm{l}$ of RNAse-free water makes a $10 \mu \mathrm{M}$ solution in a $10 \mu \mathrm{M}$ Tris- $\mathrm{HCl}, \mathrm{pH} 8.0,20 \mathrm{mM} \mathrm{NaCl}, 1 \mathrm{mM}$ EDTA buffered solution.

## APPLICATIONS

C4 siRNA ( m ) is recommended for the inhibition of C4 expression in mouse 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 $10 \mu \mathrm{M}$ in $66 \mu \mathrm{l}$. 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

C4 $\alpha$ (C-2): sc-271181 is recommended as a control antibody for monitoring of C 4 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-IgGк BP-HRP: sc-516102 or m-IgGк BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz ${ }^{\circledR}$ Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-lgGк BP-FITC: sc-516140 or m-lgGк BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz ${ }^{\circledR}$ Mounting Medium: sc-24941 or UltraCruz ${ }^{\circledR}$ Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor C4 gene expression knockdown using RT-PCR Primer: C4 (m)-PR: sc-42845-PR (20 $\mu \mathrm{l})$. Annealing temperature for the primers should be $55-60^{\circ} \mathrm{C}$ and the extension temperature should be $68-72^{\circ} \mathrm{C}$.

## 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.

