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C4BP β siRNA (h): sc-42741

BACKGROUND

The complement component proteins C3, C4 and C5 are potent anaphylatoxins that are released during classical complement activation, a system of ligand-surface protein interactions that aid in the elimination of pathogens. These proteins belong to the α 2-Macroglobulin family, but retain distinctive features including an anaphylatoxin domain and a netrin (NTR) domain. They are also expressed as single-chain precursors, which are cleaved into α , β and γ subunits that are linked by disulfide bonds. Complement C4 is an essential component for the activation of the complement pathway, which acts through the receptor CR1 (CD35). Complement C4 is predominately expressed in liver and its precursor contains C4a anaphylatoxin and C4b. The full length C4 protein is cleaved into an α chain, a β chain and a γ chain. C4 exists as two functionally distinct isotypes, C4A and C4B, which react preferentially with amino groups and hydroxyl groups, respectively. Excessive complement activation by C4 is negatively regulated by C4BP (C4 binding protein), a fluid-phase complement inhibitor that protects against complement-induced cell apoptosis. The C4BP complex contains α and β chains which act together to accelerate inactivation of C4, thereby controlling the classical pathway of complement activation.

REFERENCES

- Scharfstein, J., et al. 1978. Human C4-binding protein. I. Isolation and characterization. *J. Exp. Med.* 148: 207-222.
- Chung, L.P., et al. 1985. Molecular cloning and characterization of the cDNA coding for C4b-binding protein, a regulatory protein of the classical pathway of the human complement system. *Biochem. J.* 230: 133-141.
- Blom, A.M., et al. 1999. A cluster of positively charged amino acids in the C4BP α -chain is crucial for C4b binding and factor I cofactor function. *J. Biol. Chem.* 274: 19237-19245.
- Online Mendelian Inheritance in Man, OMIM™. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 120830. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
- Blom, A.M., et al. 2000. Positively charged amino acids at the interface between α -chain CCP1 and CCP2 of C4BP are required for regulation of the classical C3-convertase. *Mol. Immunol.* 37: 445-453.

CHROMOSOMAL LOCATION

Genetic locus: C4BPB (human) mapping to 1q32.2.

PRODUCT

C4BP β 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 C4BP β shRNA Plasmid (h): sc-42741-SH and C4BP β shRNA (h) Lentiviral Particles: sc-42741-V as alternate gene silencing products.

For independent verification of C4BP β (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-42741A, sc-42741B and sc-42741C.

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

C4BP β siRNA (h) is recommended for the inhibition of C4BP β 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

C4BP β (E-1): sc-514553 is recommended as a control antibody for monitoring of C4BP β 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 C4BP β gene expression knockdown using RT-PCR Primer: C4BP β (h)-PR: sc-42741-PR (20 μ l). 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.