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CD16 siRNA (h): sc-42758

BACKGROUND

CD16, the low affinity Fc γ receptor III (Fc γ RIII) for IgG, exists both as a peptide-anchored form known as Fc γ RIIIA or CD16-A in human natural killer cells and macrophages and as a glycosylphosphatidylinositol-anchored form (Fc γ RIIIB or CD16-B) in neutrophils. CD16-A requires association of the γ subunit of Fc ϵ RI or the ζ subunit of the TCR-CD3 complex for cell surface expression. CD16-B is polymorphic; the two alleles are designated NA1 and NA2. CD16 is one of only four eukaryotic receptors known to exist natively in both the transmembrane (TM) isoform (CD16-A) and glycosylphosphatidylinositol (GPI) isoform (CD16-B). Patients with paroxysmal nocturnal haemoglobinuria (PNH) have only about 10% of the normal levels of CD16 on their neutrophils, whereas the expression of FcRIII is unaffected. Analysis of FcRIII expression in cells of PNH patients, known to be deficient in PI-linked proteins, suggests FcRIII is not PI-linked in monocytes.

REFERENCES

1. Fleit, H.B., et al. 1982. Human neutrophil Fc γ receptor distribution and structure. Proc. Natl. Acad. Sci. USA 79: 3275-3279.
2. Perussia, B., et al. 1984. The Fc receptor for IgG on human natural killer cells: phenotypic, functional, and comparative studies with monoclonal antibodies. J. Immunol. 133: 180-189.
3. Huizinga, T.W., et al. 1988. The PI-linked receptor FcRIII is released on stimulation of neutrophils. Nature 333: 667-669.
4. Nagarajan, S., et al. 1995. Ligand binding and phagocytosis by CD16 (Fc γ receptor III) isoforms. Phagocytic signaling by associated ζ and γ subunits in Chinese hamster ovary cells. J. Biol. Chem. 270: 25762-25770.

CHROMOSOMAL LOCATION

Genetic locus: FCGR3A/FCGR3B (human) mapping to 1q23.3.

PRODUCT

CD16 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 CD16 shRNA Plasmid (h): sc-42758-SH and CD16 shRNA (h) Lentiviral Particles: sc-42758-V as alternate gene silencing products.

For independent verification of CD16 (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-42758A, sc-42758B and sc-42758C.

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

CD16 siRNA (h) is recommended for the inhibition of CD16 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

CD16 (DJ130c): sc-20052 is recommended as a control antibody for monitoring of CD16 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 CD16 gene expression knockdown using RT-PCR Primer: CD16 (h)-PR: sc-42758-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. Fernandez-Vizarra, P., et al. 2012. Immunoglobulin G Fc receptor deficiency prevents Alzheimer-like pathology and cognitive impairment in mice. Brain 135: 2826-2837.

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.