

# CD14 siRNA (m): sc-29962

## BACKGROUND

Lipopolysaccharide (LPS) elicits the secretion of mediators and cytokines produced by activated macrophages and monocytes. CD14 is a glycosylphosphatidylinositol (GPI)-anchored protein found on the surfaces of monocytes and polymorphonuclear leukocytes. CD14 functions as a receptor for LPS, resulting in the secretion of various proteins. An important component in the LPS activation of monocytes through the CD14 receptor is the "adapter molecule," lipopolysaccharide binding protein (LBP). There are two forms of CD14, a membrane-associated form (mCD14), and a soluble form (sCD14). mCD14 responds to LPS alone and facilitates the secretion of proteins, while cells not expressing mCD14 fail to respond to LPS. The cells that lack mCD14 respond to LPS/LBP in the presence of sCD14.

## REFERENCES

1. Simmons, D.L., et al. 1989. Monocyte antigen CD14 is a phospholipid anchored membrane protein. *Blood* 73: 284-289.
2. Schumann, R.R. 1992. Function of lipopolysaccharide (LPS)-binding protein (LBP) and CD14, the receptor for LPS/LBP complexes: a short review. *Res. Immunol.* 143: 11-15.
3. Kielan, T.L., et al. 1995. CD14 and other recognition molecules for lipopolysaccharide: a review. *Immunopharmacology* 29: 187-205.
4. Camussi, G., et al. 1995. Lipopolysaccharide binding protein and CD14 modulate the synthesis of platelet-activating factor by human monocytes and mesangial and endothelial cells stimulated with lipopoly-saccharide. *J. Immunol.* 155: 316-324.
5. Detmers, P.A., et al. 1995. Endotoxin receptors (CD14) are found with CD16 (Fc  $\gamma$  RIII) in an intracellular compartment of neutrophils that contains alkaline phosphatase. *J. Immunol.* 155: 2085-2095.

## CHROMOSOMAL LOCATION

Genetic locus: Cd14 (mouse) mapping to 18 B2.

## PRODUCT

CD14 siRNA (m) 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CD14 shRNA Plasmid (m): sc-29962-SH and CD14 shRNA (m) Lentiviral Particles: sc-29962-V as alternate gene silencing products.

For independent verification of CD14 (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-29962A, sc-29962B and sc-29962C.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

CD14 shRNA (m) Lentiviral Particles is recommended for the inhibition of CD14 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  $\mu$ M in 66  $\mu$ 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

CD14 (H-4): sc-515785 is recommended as a control antibody for monitoring of CD14 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 CD14 gene expression knockdown using RT-PCR Primer: CD14 (m)-PR: sc-29962-PR (20  $\mu$ l, 401 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.