CD14 shRNA (h) Lentiviral Particles: sc-29248-V

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

CHROMOSOMAL LOCATION
Genetic locus: CD14 (human) mapping to 5q31.3.

PRODUCT
CD14 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 4 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10^6 infectious units of virus (IFU) in Dulbecco’s Modified Eagle’s Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see CD14 siRNA (h): sc-29248 and CD14 shRNA Plasmid (h): sc-29248-SH as alternate gene silencing products.

STORAGE
Store lentiviral particles at -80°C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS
CD14 shRNA (h) Lentiviral Particles is recommended for the inhibition of CD14 expression in human cells.

SUPPORT REAGENTS
Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING
CD14 (IA3B11B5): sc-58951 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-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-PE (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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 (h)-PR: sc-29248-PR (20 µl, 586 bp). Annealing temperature for the primers should be 55-60°C and the extension temperature should be 68-72°C.

BIOSAFETY
Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE
The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS
See our web site at www.scbt.com for detailed protocols and support products.