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TLR5 siRNA (r): sc-270186

BACKGROUND

Six human homologs of the *Drosophila* Toll receptor were initially identified based on their sequence similarities and designated Toll-like receptors (TLR). Toll receptors are involved in mediating dorsoventral polarization in the developing *Drosophila* embryo and also participate in the host immunity. The TLR family of proteins are characterized by a highly conserved Toll homology (TH) domain, which is essential for Toll-induced signal transduction. TLR1, as well as the other TLR family members, are type I transmembrane receptors that characteristically contain an extracellular domain consisting of several leucine-rich regions along with a single cytoplasmic Toll/IL-1R-like domain. TLR2 and TLR4 are activated in response to lipopolysaccharide (LPS) stimulation, which results in the activation and translocation of NF κ B and suggests that these receptors are involved in mediating inflammatory responses. TLR5 specifically participates in the innate immune response to microbial agents. TLR5 is highly expressed in ovary and in peripheral blood leukocytes, most abundantly in monocytes and to a lesser extent in prostate and testis.

REFERENCES

1. Gay, N.J., et al. 1991. *Drosophila* Toll and IL-1 receptor. *Nature* 351: 355-356.
2. Medzhitov, R., et al. 1997. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 388: 394-397.
3. Rock, F.L., et al. 1998. A family of human receptors structurally related to *Drosophila* Toll. *Proc. Natl. Acad. Sci. USA* 95: 588-593.
4. Yang, R.B., et al. 1998. Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. *Nature* 395: 284-288.
5. Brightbill, H.D., et al. 1999. Host defense mechanisms triggered by microbial lipoproteins through Toll-like receptors. *Science* 285: 732-736.
6. Chow, J.C., et al. 1999. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J. Biol. Chem.* 274: 10689-10692.
7. Schwandner, R., et al. 1999. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by Toll-like receptor 2. *J. Biol. Chem.* 274: 17406-17409.
8. Takeuchi, O., et al. 1999. TLR6: a novel member of an expanding Toll-like receptor family. *Gene* 231: 59-65.

CHROMOSOMAL LOCATION

Genetic locus: Tlr5 (rat) mapping to 13q26.

PRODUCT

TLR5 siRNA (r) 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 TLR5 shRNA Plasmid (r): sc-270186-SH and TLR5 shRNA (r) Lentiviral Particles: sc-270186-V as alternate gene silencing products.

For independent verification of TLR5 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270186A, sc-270186B and sc-270186C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

TLR5 siRNA (r) is recommended for the inhibition of TLR5 expression in rat 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

TLR5 (H-11): sc-518106 is recommended as a control antibody for monitoring of TLR5 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 MarkerTM 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 TLR5 gene expression knockdown using RT-PCR Primer: TLR5 (r)-PR: sc-270186-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ 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.