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# TLR4 siRNA (r): sc-156001

## 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 $\kappa$ B and suggests that these receptors are involved in mediating inflammatory responses. Expression of TLR receptors is highest in peripheral blood leukocytes, macrophages, and monocytes. TLR6 is highly homologous to TLR1, sharing greater than 65% sequence identity, and, like other members of TLR family, it induces NF $\kappa$ B signaling upon activation.

## 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.

## CHROMOSOMAL LOCATION

Genetic locus: Tlr4 (rat) mapping to 5q24.

## PRODUCT

TLR4 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TLR4 shRNA Plasmid (r): sc-156001-SH and TLR4 shRNA (r) Lentiviral Particles: sc-156001-V as alternate gene silencing products.

For independent verification of TLR4 (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-156001A, sc-156001B and sc-156001C.

## 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

TLR4 siRNA (r) is recommended for the inhibition of TLR4 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  $\mu$ 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

TLR4 (25): sc-293072 is recommended as a control antibody for monitoring of TLR4 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).

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TLR4 gene expression knockdown using RT-PCR Primer: TLR4 (r)-PR: sc-156001-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Yang, J., et al. 2012. High mobility group box-1 induces migration of vascular smooth muscle cells via TLR4-dependent PI3K/Akt pathway activation. *Mol. Biol. Rep.* 39: 3361-3367.
2. Nair, A.R., et al. 2015. Angiotensin II-induced hypertensive renal inflammation is mediated through HMGB1-TLR4 signaling in rat tubulo-epithelial cells. *Exp. Cell Res.* 335: 238-247.
3. Han, Y., et al. 2016. Cardiac Troponin I exacerbates myocardial ischaemia/reperfusion injury by inducing the adhesion of monocytes to vascular endothelial cells via a TLR4/NF $\kappa$ B-dependent pathway. *Clin. Sci.* 130: 2279-2293.
4. Li, F., et al. 2018. NLRP3 deficiency accelerates pressure overload-induced cardiac remodeling via increased TLR4 expression. *J. Mol. Med.* 96: 1189-1202.
5. Qian, J., et al. 2019. Chronic morphine-mediated upregulation of high mobility group box 1 in the spinal cord contributes to analgesic tolerance and hyperalgesia in rats. *Neurotherapeutics* 17: 722-742.
6. Gao, J., et al. 2020. Neuroprotective effects of trilobatin, a novel naturally occurring Sirt3 agonist from *Lithocarpus polystachyus* Rehd., mitigate cerebral ischemia/reperfusion injury: involvement of TLR4/NF $\kappa$ B and Nrf2/Keap-1 signaling. *Antioxid. Redox Signal.* 33: 117-143.
7. Li, H.Y., et al. 2020. Investigation and comparison of the protective activities of three functional proteins-lactoferrin,  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin-in cerebral ischemia reperfusion injury. *J. Dairy Sci.* 103: 4895-4906.

## RESEARCH USE

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