

# TIM-3 siRNA (h): sc-72034

## BACKGROUND

CD4<sup>+</sup> T helper lymphocytes can be divided into types 1 (Th1) and 2 (Th2) on the basis of their cytokine secretion patterns. Th1 cells and their associated cytokines are involved in cell-mediated immunity to intracellular pathogens and delayed-type hypersensitivity reactions. Th2 cells are involved in the control of extracellular helminthic infections and the promotion of atopic and allergic diseases. T cell Ig- and Mucin domain-containing molecules (TIMs) are a family of molecules expressed on T cells. TIM-3 is a Th1-specific cell surface protein that regulates macrophage activation and enhances the severity of experimental autoimmune encephalomyelitis in mice. TIM-3 binds galectin-9, thereby inducing apoptosis of Th1 cells. This interaction with galectin-9 also stimulates the phosphorylation of TIM-3 on residue Y265 by the interleukin-inducible T cell kinase, Emt.

## REFERENCES

1. Monney, L., et al. 2002. Th1-specific cell surface protein TIM-3 regulates macrophage activation and severity of an autoimmune disease. *Nature* 415: 536-541.
2. Sanchez-Fueyo, A., et al. 2003. TIM-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance. *Nat. Immunol.* 4: 1093-1101.
3. Sabatos, C.A., et al. 2003. Interaction of TIM-3 and TIM-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. *Nat. Immunol.* 4: 1102-1110.
4. Online Mendelian Inheritance in Man, OMIM™. 2003. Johns Hopkins University, Baltimore, MD. MIM Number: 606652. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

## CHROMOSOMAL LOCATION

Genetic locus: HAVCR2 (human) mapping to 5q33.3.

## PRODUCT

TIM-3 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TIM-3 shRNA Plasmid (h): sc-72034-SH and TIM-3 shRNA (h) Lentiviral Particles: sc-72034-V as alternate gene silencing products.

For independent verification of TIM-3 (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-72034A, sc-72034B and sc-72034C.

## 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

TIM-3 siRNA (h) is recommended for the inhibition of TIM-3 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  $\mu$ 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

TIM-3 (182H2B): sc-517632 is recommended as a control antibody for monitoring of TIM-3 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 TIM-3 gene expression knockdown using RT-PCR Primer: TIM-3 (h)-PR: sc-72034-PR (20  $\mu$ l, 513 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Zhang, Y., et al. 2012. Tim-3 regulates pro- and anti-inflammatory cytokine expression in human CD14<sup>+</sup> monocytes. *J. Leukoc. Biol.* 91: 189-196.
2. Yu, M., et al. 2017. Interference with Tim-3 protein expression attenuates the invasion of clear cell renal cell carcinoma and aggravates anoikis. *Mol. Med. Rep.* 15: 1103-1108.
3. Chiou, B., et al. 2018. Semaphorin4A and H-ferritin utilize Tim-1 on human oligodendrocytes: a novel neuro-immune axis. *Glia* 66: 1317-1330.

## 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.