

T-bet siRNA (r): sc-270589

BACKGROUND

T helper (Th) lymphocytes differentiate into two unique subsets, Th1 and Th2, which differ both in function and in the cytokines they secrete. Th1 and Th2 cytokines promote the growth and differentiation of their subset, and inhibit the growth and differentiation of the opposing subset. T-bet (T box expressed in T cells) is a Th1-specific T box transcription factor that controls the expression of the Th1 cytokine, IFN- γ . T-bet also converts effector Th2 cells into the opposing Th1 subset. T-bet is selectively expressed in Th1 cells. The level of T-bet expression is increased by signals mediated by the T cell receptor (TCR). IL-12 also induces an increase in the level of T-bet. T-bet was originally isolated from nuclear extracts of resting and PMA/ionomycin-activated AE7 cells. T-bet is expressed in low levels in AE7 cells, and in increased levels in stimulated AE7.

REFERENCES

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4. O'Garra, A. 1998. Checkpoints for regulation of development and interferon γ production by Th1 cells in TCR-transgenic models. *Eur. Cytokine Netw.* 9: 41-42.
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6. Mehta, D.S., et al. 2005. NFATc2 and T-bet contribute to T-helper-cell-subset-specific regulation of IL-21 expression. *Proc. Natl. Acad. Sci. USA* 102: 2016-2021.
7. Tong, Y., et al. 2005. T-bet antagonizes mSin3a recruitment and transactivates a fully methylated IFN- γ promoter via a conserved T-box half-site. *Proc. Natl. Acad. Sci. USA* 102: 2034-2039.
8. Dorfman, D.M., et al. 2005. T-bet, a T cell-associated transcription factor, is expressed in Hodgkin's lymphoma. *Hum. Pathol.* 36: 10-15.

CHROMOSOMAL LOCATION

Genetic locus: Tbx21 (rat) mapping to 10q31.

PRODUCT

T-bet 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 T-bet shRNA Plasmid (r): sc-270589-SH and T-bet shRNA (r) Lentiviral Particles: sc-270589-V as alternate gene silencing products.

For independent verification of T-bet (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-270589A, sc-270589B and sc-270589C.

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 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

T-bet siRNA (r) is recommended for the inhibition of T-bet 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

T-bet (4B10): sc-21749 is recommended as a control antibody for monitoring of T-bet 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 Marker™ 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 T-bet gene expression knockdown using RT-PCR Primer: T-bet (r)-PR: sc-270589-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.