

TOX siRNA (h): sc-77552

BACKGROUND

TOX (thymocyte selection-associated high mobility group (HMG) box protein) is a 526 amino acid nuclear protein that is a member of the HMG box family of DNA-binding proteins and likely plays a role in the regulation of T-cell development. Expression of TOX is upregulated by pre-T cell receptor (pre-TCR) and TCR activation in immature thymocytes, but not by TCR activation in mature thymocytes. CD4 T cells fail to develop in TOX-deficient mice, however functional CD8⁺ T cells still develop, suggesting that TOX-dependent transition to the CD4⁺CD8 stage is required for development of class II major histocompatibility complex-specific T cells. Calcineurin activation events and CD8 lineage commitment seem to be linked due to evidence that up-regulation of TOX in double positive thymocytes is calcineurin dependent.

REFERENCES

1. Saito, T., et al. 1998. Positive and negative thymocyte selection. *Crit. Rev. Immunol.* 18: 359-370.
2. Mitnacht, R., et al. 1998. Opposite CD4/CD8 lineage decisions of CD4⁺ mouse and rat thymocytes to equivalent triggering signals: correlation with thymic expression of a truncated CD8- α chain in mice but not rats. *J. Immunol.* 160: 700-707.
3. Wilkinson, B., et al. 2002. TOX: an HMG box protein implicated in the regulation of thymocyte selection. *Nat. Immunol.* 3: 272-280.
4. Aliahmad, P., et al. 2004. TOX provides a link between calcineurin activation and CD8 lineage commitment. *J. Exp. Med.* 199: 1089-1099.
5. Laky, K., et al. 2005. Receptor signals and nuclear events in CD4 and CD8 T cell lineage commitment. *Curr. Opin. Immunol.* 17: 116-121.
6. Aliahmad, P., et al. 2006. Commitment issues: linking positive selection signals and lineage diversification in the thymus. *Immunol. Rev.* 209: 253-273.
7. Laky, K., et al. 2006. TCR and Notch signaling in CD4 and CD8 T-cell development. *Immunol. Rev.* 209: 274-283.

CHROMOSOMAL LOCATION

Genetic locus: TOX (human) mapping to 8q12.1.

PRODUCT

TOX 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TOX shRNA Plasmid (h): sc-77552-SH and TOX shRNA (h) Lentiviral Particles: sc-77552-V as alternate gene silencing products.

For independent verification of TOX (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-77552A, sc-77552B and sc-77552C.

PROTOCOLS

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

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

TOX siRNA (h) is recommended for the inhibition of TOX 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 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

TOX (H-2): sc-374137 is recommended as a control antibody for monitoring of TOX 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 TOX gene expression knockdown using RT-PCR Primer: TOX (h)-PR: sc-77552-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.