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# TdT shRNA (h) Lentiviral Particles: sc-44143-V

## BACKGROUND

Terminal deoxynucleotidyltransferase (TdT) is a DNA polymerase which catalyzes the addition of deoxyribonucleotides onto the 3'-hydroxyl end of DNA primers without template direction. The enzyme thus provides a unique method for the labeling of the 3' termini of DNA. The human TdT gene maps to chromosome 10q24.1 and encodes a 510 amino acid protein. Human TdT is synthesized as a single chain peptide that elicits a minor preference for incorporation of deoxyribonucleotides over ribonucleotides forming DNA strands. TdT is present in immature thymocytes, some bone marrow cells, transformed pre-B and pre-T cell lines, and leukemia cells.

## REFERENCES

1. Bentolila, L.A., et al. 1997. Constitutive expression of terminal deoxynucleotidyl transferase in transgenic mice is sufficient for N region diversity to occur at any Ig locus throughout B cell differentiation. *J. Immunol.* 158: 715-723.
2. Marshall, A.J., et al. 1998. Terminal deoxynucleotidyl transferase expression during neonatal life alters D(H) reading frame usage and Ig-receptor-dependent selection of V regions. *J. Immunol.* 161: 6657-6663.
3. Nourrit, F., et al. 1999. Methylation of the promoter region may be involved in tissue-specific expression of the mouse terminal deoxynucleotidyl transferase gene. *J. Mol. Biol.* 292: 217-227.
4. Aono, A., et al. 2000. Forced expression of terminal deoxynucleotidyl transferase in fetal thymus resulted in a decrease in  $\gamma/\delta$  T cells and random dissemination of Vg3Vd1 T cells in skin of newborn but not adult mice. *Immunology* 99: 489-497.
5. Feeney, A.J., et al. 2001. Terminal deoxynucleotidyl transferase deficiency decreases autoimmune disease in MRL-Fas(lpr) mice. *J. Immunol.* 167: 3486-3493.
6. Boule, J.B., et al. 2001. Terminal deoxynucleotidyl transferase indiscriminately incorporates ribonucleotides and deoxyribonucleotides. *J. Biol. Chem.* 276: 31388-31393.

## CHROMOSOMAL LOCATION

Genetic locus: DNTT (human) mapping to 10q24.1.

## PRODUCT

TdT shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see TdT siRNA (h): sc-44143 and TdT shRNA Plasmid (h): sc-44143-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

TdT shRNA (h) Lentiviral Particles is recommended for the inhibition of TdT expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

TdT (C-11): sc-393710 is recommended as a control antibody for monitoring of TdT 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TdT gene expression knockdown using RT-PCR Primer: TdT (h)-PR: sc-44143-PR (20  $\mu$ l, 426 bp). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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