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SANTA CRUZ BIOTECHNOLOGY, INC.

TdT siRNA (h): sc-44143

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 γ/δ 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 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TdT shRNA Plasmid (h): sc-44143-SH and TdT shRNA (h) Lentiviral Particles: sc-44143-V as alternate gene silencing products.

For independent verification of TdT (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-44143A, sc-44143B and sc-44143C.

RESEARCH USE

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

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

TdT siRNA (h) is recommended for the inhibition of TdT 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 µM in 66 µl. 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

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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 TdT gene expression knockdown using RT-PCR Primer: TdT (h)-PR: sc-44143-PR (20 µl, 426 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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