SANTA CRUZ BIOTECHNOLOGY, INC.

Thyroperoxidase siRNA (h): sc-61684

BACKGROUND

The synthesis of thyroid hormones is an oxidative process that produces reactive oxygen species and requires Thyroperoxidase (TPO), a hemoprotein that is one of the major autoantigens involved in autoimmune thyroid diseases. Thyroperoxidase is a 933 amino acid, type I transmembrane glycoprotein that plays a key role in thyroid hormone synthesis and autoimmunity. TPO catalyzes the iodination of proteins, therefore causing iodide retention within thyroid cells. The ecto-domain of Thyroperoxidase includes a large N-terminal myeloperoxidase-like domain, followed by a complement control protein domain and an epidermal growth factor-like domain. Thyroperoxidase also mediates the organification and intracellular retention of radioiodide, which may lead to rapid tumor cell death. Mutations of the Thyroperoxidase gene commonly lead to goitrous congenital hypothyroidism, the most severe and frequent abnormality in thyroid iodide organification defect (IOD), in which iodide in the thyroid gland cannot be oxidized and/or bound to the protein.

REFERENCES

1. Fayadat, L., et al. 1998. Human thyroperoxidase is largely retained and rapidly degraded in the endoplasmic reticulum. Its N-glycans are required for folding and intracellular trafficking. Endocrinology 139: 4277-4285.
2. Fayadat, L., et al. 2000. Degradation of human thyroperoxidase in the endoplasmic reticulum involves two different pathways depending on the folding state of the protein. J. Biol. Chem. 275: 15948-15954.
3. Huang, M., et al. 2001. Ectopic expression of the Thyroperoxidase gene augments radioiodide uptake and retention mediated by the sodium iodide symporter in non-small cell lung cancer. Cancer Gene Ther. 8: 612-628.
4. Blanchin, S., et al. 2003. Complement activation by direct C4 binding to Thyroperoxidase in Hashimoto's thyroiditis. Endocrinology 144: 5422-5429.
5. Ferrand, M., et al. 2003. Increasing diversity of human thyroperoxidase generated by alternative splicing. Characterized by molecular cloning of new transcripts with single- and multispliced mRNAs. J. Biol. Chem. 278: 3793-3800.
6. Fernandez Romero, D.S., et al. 2005. Chronic urticaria with alterations of the thyroid function and thyroid peroxidase antibodies. Medicina 65: 231-234.

CHROMOSOMAL LOCATION

Genetic locus: TPO (human) mapping to 2p25.3.

PRODUCT

Thyroperoxidase 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 Thyroperoxidase shRNA Plasmid (h): sc-61684-SH and Thyroperoxidase shRNA (h) Lentiviral Particles: sc-61684-V as alternate gene silencing products.

For independent verification of Thyroperoxidase (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-61684A, sc-61684B and sc-61684C.

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

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

Thyroperoxidase (A-5): sc-376876 is recommended as a control antibody for monitoring of Thyroperoxidase 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 Thyroperoxidase gene expression knockdown using RT-PCR Primer: Thyroperoxidase (h)-PR: sc-61684-PR (20 µl). 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.