

Human thyroglobulin

Thyroglobulin is a glycoprotein that is produced exclusively by the follicular cells of the thyroid. It is secreted into the follicular lumen, where it serves as a storage reservoir for the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Thyroglobulin is a protein dimer of 660 kDa in size, it has a high number (approximately 60) of disulfide bonds per monomer and 17 glycosylation sites (1). The production of thyroglobulin is stimulated by thyroid-stimulating hormone (TSH), intrathyroidal iodine deficiency, and the presence of thyroid-stimulating immunoglobulins.

Small amounts of intact thyroglobulin are secreted and detectable in the serum of healthy individuals. The concentration of serum thyroglobulin increases substantially due to follicular destruction through inflammation or rapid disordered growth of thyroid tissue (2).

In clinical practice, the primary use of serum thyroglobulin measurements occurs in the follow-up of patients with differentiated thyroid cancer (DTC) after total thyroidectomy and radioactive iodine ablation (3, 4). Thyroglobulin measurement is not recommended for the screening or initial diagnosis of thyroid cancer due to the overlap of thyroglobulin levels in patients with benign thyroid diseases and DTC.

Thyroglobulin is potentially autoantigenic and elevated serum concentrations of thyroglobulin autoantibodies are found in subjects with autoimmune thyroid diseases (AITD) (5). Graves' disease and Hashimoto's thyroiditis are the two most frequent clinical presentations of AITD diagnosis. A negative thyroglobulin antibody result can help in terms of excluding the diagnosis of Hashimoto's thyroiditis. However,

a positive antibody result is not yet recommended to be used for the diagnosis of Hashimoto's thyroiditis. This is because thyroglobulin antibodies can also be found in other conditions (6, 7).

In addition, thyroglobulin antibodies are often measured to authenticate that thyroglobulin measurement has not been compromised (8, 9). Thyroglobulin antibodies can bind the circulating thyroglobulin and, therefore, interfere with the measurement of thyroglobulin. This can cause falsely low or undetectable levels of thyroglobulin. It is worth mentioning here that thyroglobulin and thyroglobulin antibodies show mutual interference in their immunoassays.

Reagents for immunoassay development

Hytest provides monoclonal antibodies (MAbs) for the development of highly sensitive, quantitative thyroglobulin immunoassays. We offer both native human thyroglobulin and recombinant human thyroglobulin expressed in a mammalian cell line to be used for the development of assays for the detection of thyroglobulin autoantibodies. These antigens can also be used as calibrators for thyroglobulin immunoassays.

CLINICAL UTILITY

- Differentiated thyroid diseases (DTC)
- Autoimmune thyroid diseases (AITD)

Native thyroglobulin is purified from thyroid glands to homogeneity by salting out with ammonium sulfate and gel filtration using Sephacryl S-200. The purity of the protein is >90 % (Fig. 1A). A few visible protein bands belong to thyroglobulin since they are recognized by specific monoclonal antibodies in Western blotting. Recombinant thyroglobulin is a full-sized subunit of human thyroglobulin (amino acid residues 1-2768 UniProtKB P01266 accession number) containing C-terminal GlyAlaProGly4SerHis10-tag. The protein is purified to homogeneity using metal affinity chromatography. The protein presentation with 5.4% sucrose is optimized for storage in lyophilized form. The purity of the protein is >95% (Fig. 1B).

Immunochemical properties of human recombinant thyroglobulin were analyzed in comparison with native human thyroglobulin purified from human thyroid glands. Serum samples of patients with various autoimmune thyroid diseases were tested with both native and recombinant thyroglobulin preparations used as antigens for plate coating. As is the case in Fig. 2, native and recombinant preparations of thyroglobulin have very similar immunoreactivity towards thyroglobulin autoantibodies (correlation coefficient of 0.99 (n=23)).

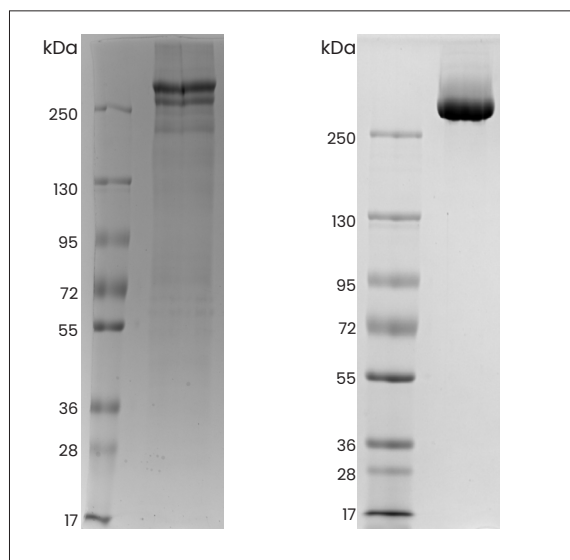


Figure 1.
SDS-PAGE (5-15%) analysis of purified human thyroglobulin in reducing conditions.

- A. Native thyroglobulin from human thyroid gland, 3 µg.
B. Recombinant thyroglobulin expressed in mammalian cell line, 4 µg.

Native and recombinant human thyroglobulin can be utilized as a calibrator in sandwich immunoassays for the detection of thyroglobulin in serum or plasma samples. As is the case in Fig. 3, both native and recombinant thyroglobulin are immunochemically active in the sandwich immunoassay.

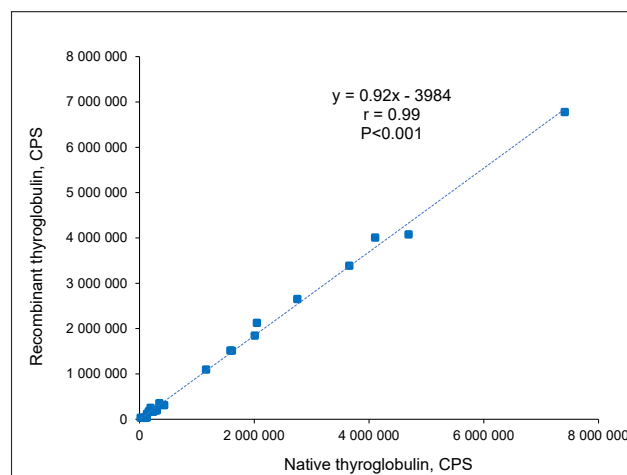


Figure 2.
Scatter plot of fluorescent intensities for native human thyroglobulin and recombinant human thyroglobulin used as antigens for plate coating tested with serum samples from patients with AITD.
Immunoassay plates were coated with native or recombinant thyroglobulin (0.1 µg/well). Serum samples were diluted 1/50 and incubated in wells for 30 min. Autoantibodies were detected with anti-human IgG antibodies labelled with stable Eu³⁺ chelate. Fluorescent signal is expressed in CPS.

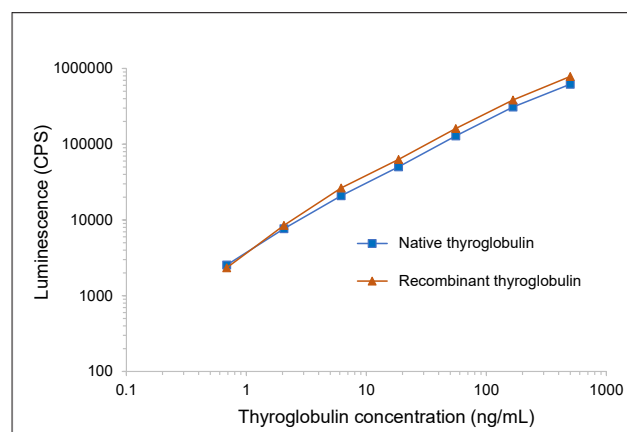


Figure 3.
Calibration curve with native and recombinant thyroglobulin for the 5F9cc-5E6cc (capture-detection MABs) sandwich chemiluminescent immunoassay. A mixture of capture antibodies labelled with biotin, antigen, and detection antibodies labelled with alkaline phosphatase was incubated for 15 minutes at 37 °C in immunoassay microplate wells covered with streptavidin. A luminescent signal is expressed in CPS.

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Thyroglobulin	2TG12cc	5E6cc	IgG2b	<i>In vitro</i> , EIA
		5F9cc	IgG2a	<i>In vitro</i> , EIA, IHC

ANTIGENS

Product name	Cat. #	Purity	Source
Thyroglobulin	8TG52	>90%	Human thyroid gland
Thyroglobulin, human, recombinant	8RTG4	>95%	Recombinant