Human thyroglobulin and thyroglobulin antibodies

Thyroglobulin is a glycoprotein that is produced exclusively by the follicular cells of the thyroid. It 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).

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 (2, 3). 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. Elevated serum concentrations of thyroglobulin autoantibodies are found in subjects with autoimmune thyroid diseases (AITD) (4). 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 autoantibodies can also be found in other conditions (5, 6).

In addition, thyroglobulin autoantibodies are often measured to authenticate that thyroglobulin measurement has not been compromised (7, 8). 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.

NATIVE AND RECOMBINANT THYROGLOBULIN

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.

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 % (see Figure 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 the 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% (see Figure 1B).

CLINICAL UTILITY

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

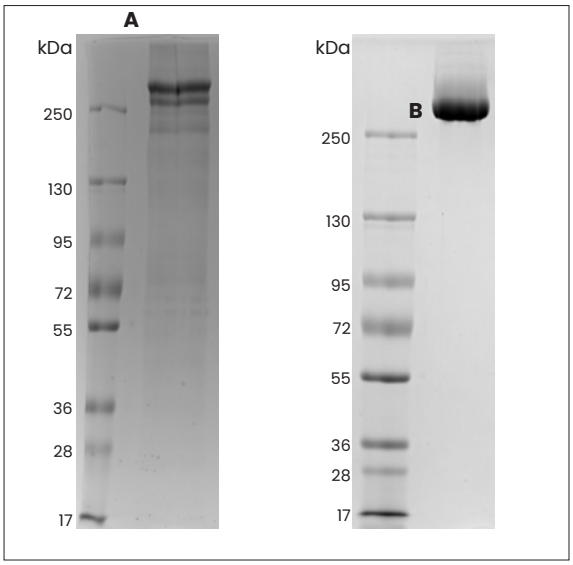

Figure 1.
SDS-PAGE (5-15%) analysis of purified human thyroglobulin in reducing conditions. A. Native thyroglobulin from human thyroid gland, 3 µg. B. Recombinant thyroglobulin expressed in mammalian cell line, 4 µg.

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 shown in Figure 2, native and recombinant preparations of thyroglobulin have very similar immunoreactivity towards thyroglobulin auto-antibodies (correlation coefficient of 0.99 (n=23)).

Native and recombinant human thyroglobulin can be utilized as a calibrator in immunoassays for the detection of thyroglobulin in serum or plasma samples. As is shown in Figure 3, both native and recombinant thyroglobulin are immunochemically active in the sandwich immunoassay based on the MAb pair TG16-TG12.

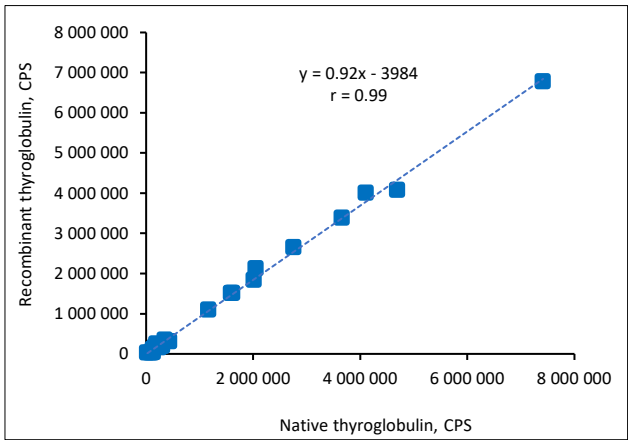

Figure 2.
Scatter plot of fluorescent intensities for native human thyroglobulin and recombinant human thyroglobulin used as antigens for plate coating tested with serum samples from patients with AITD. Immunoassay plates were coated with native or recombinant thyroglobulin (0.1 µg/well). Serum samples were diluted 1/50 and incubated in wells for 30 min. Autoantibodies were detected with anti-human IgG antibodies labelled with stable Eu^{3+} chelate. Fluorescent signal is expressed in CPS.

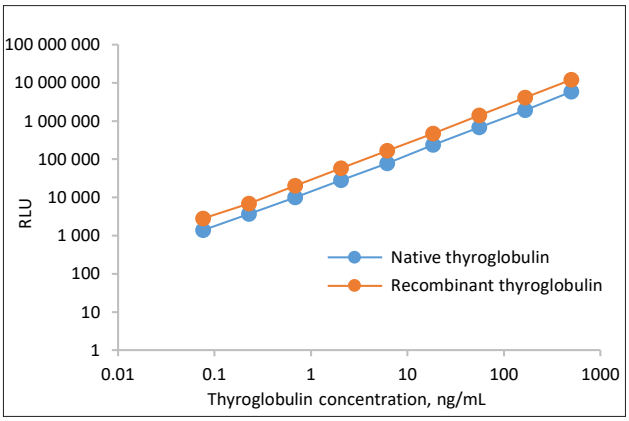

Figure 3.
Calibration curve with native and recombinant thyroglobulin for the TG16-TG12 immunoassay. A mixture of capture antibodies labelled with biotin, antigen, and detection antibodies labelled with alkaline phosphatase was incubated for 15 minutes at 37°C. The luminescent signal is expressed in RLU.

MONOCLONAL ANTIBODIES FOR THYROGLOBULIN IMMUNOASSAY DEVELOPMENT

Hytest offers different MAb combinations, which allow for the development of highly specific, sensitive, and rapid immunoassays. These immunoassays are suitable for the quantitative measurement of thyroglobulin in human serum or plasma samples. Table 1 shows the recommended antibody pair combinations for the development of thyroglobulin immunoassays.

Representative calibration curves for immunoassays based on the MAb pairs TG47-TG64 and TG16-TG12 are presented in Figure 4. Other antibody pairs that are listed above also exhibit high sensitivity (LoD ≤ 0.02 ng/mL in chemiluminescence immunoassays).

Table 1.
Recommended MAb pairs for thyroglobulin immunoassays.

Capture	Detection
TG47	TG16
TG47	TG64
TG16	TG12
TG16	TG33
TG14	TG23
TG64	TG23
TG64	TG16

Capture	Detection
TG23	TG12
5F9cc	5E6cc
TG37	TG16
TG46	TG12
TG64	TG46
TG37	TG64

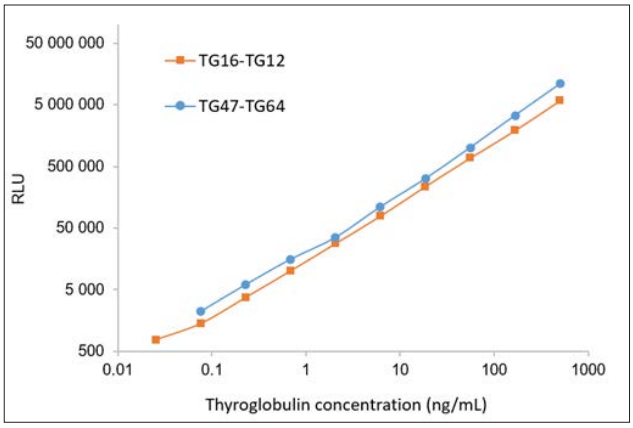

Figure 4.
Calibration curves for immunoassays TG47-TG64 and TG16-TG12. A mixture of capture antibodies labelled with biotin, antigen, and detection antibodies labelled with alkaline phosphatase was incubated for 15 minutes at 37°C. The luminescent signal is expressed in Relative Light Units (RLU).

Low interference with thyroglobulin autoantibodies

The presence of autoantibodies in serum or plasma samples may result in falsely low reported thyroglobulin levels due to interference from autoantibodies. To avoid this type of interference in thyroglobulin immunoassays, all of the developed MAbs were tested with serum samples that contained high levels of thyroglobulin autoantibodies in order to select those MAbs with either no or relatively low interference from thyroglobulin autoantibodies.

Detection of thyroglobulin in clinical samples

Thyroglobulin assays based on the recommended MAb pairs showed a high correlation with the widely used commercially available Roche Elecsys TG II assay. Thyroglobulin concentrations were measured in 50 serum samples using immunoassays based on the MAb pairs TG47-TG64 and TG16-TG12 in comparison with the Roche Elecsys TG II assay. As outlined in Figure 5, the results of the measurements obtained with both assays showed a high correlation with the results of measurements obtained with the Roche Elecsys TG II assay (R2=0.99 for the TG47-TG64 assay, and R2=0.96 for the TG16-TG12 assay, respectively).

MONOCLONAL ANTIBODIES FOR ANTI-THYROGLOBULIN IMMUNOASSAY DEVELOPMENT

Hytest has developed antibodies, which exhibit high sensitivity to the presence of thyroglobulin autoantibodies that suggest a common or similar epitope with autoantibodies. The assay for the quantitative determination of thyroglobulin autoantibodies is based on a competition principle: the thyroglobulin autoantibodies of the sample bind the antigen and from there the added labelled anti-thyroglobulin antibodies compete for the binding with the thyroglobulin autoantibodies. The intensity of the signal is inversely proportional to the concentration of thyroglobulin autoantibodies. Representative calibration curves for two sequential competitive assays based on the MAbs TG08 or TG61 and recombinant thyroglobulin are presented in Figure 6.

Detection of thyroglobulin antibodies in clinical samples

We analyzed 45 serum samples from patients with AITD using two sequential competitive assays. These are based on the MAbs TG08 or TG61 and recombinant thyroglobulin in comparison with the Roche Elecsys Anti-Tg assay that is also a sequential competitive immunoassay. The results are presented in Figure 7. Assays based on the MAbs TG08 or TG61 and recombinant thyroglobulin exhibited a relatively good correlation with the commercial Roche Elecsys Anti-Tg assay. Notably, using a mixture of TG08 (as a main antibody) with TG66 (as a supplemental antibody) or TG61 (as a main antibody) with TG66 (as a supplemental antibody) in a ratio of 3 to 1 resulted in an increased correlation with the Roche Elecsys anti-Tg assay (R2 > 0.9).

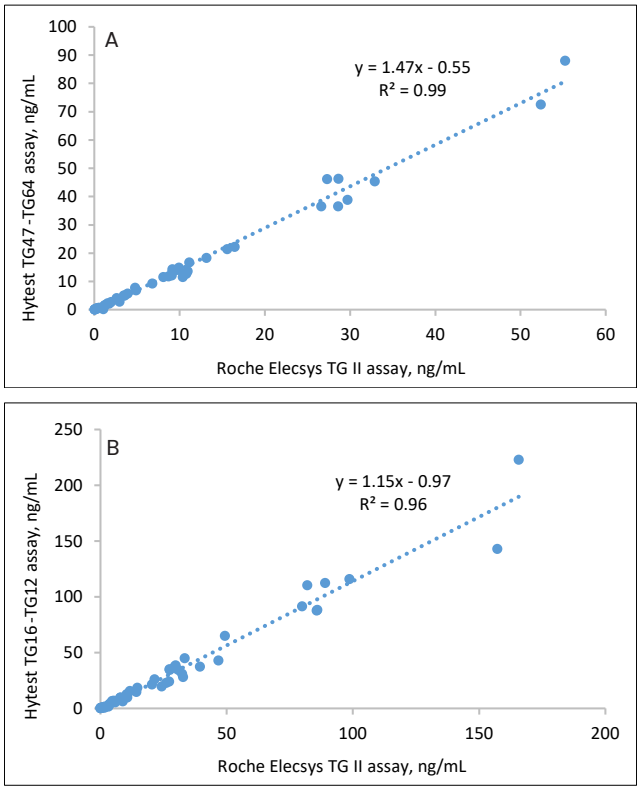

Figure 5.

A scatter plot of thyroglobulin levels measured in 50 serum samples with two immunoassays based on the MAb pairs TG47-TG64 (A) and TG16-TG12 (B) in comparison with the Roche Elecsys TG II assay. A mixture of capture antibodies labelled with biotin, calibrator, or serum samples, and detection antibodies labelled with alkaline phosphatase was incubated for 15 minutes at 37°C.

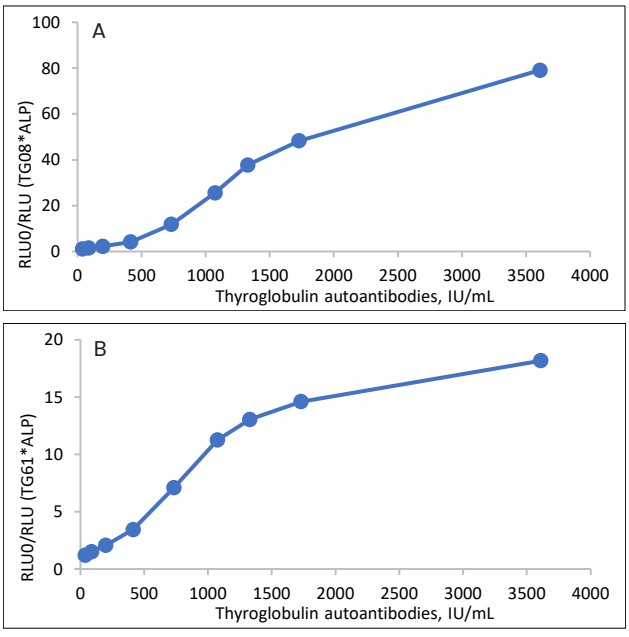

Figure 6.

Calibration curves for two sequential competitive assays based on the MAbs TG08 (A) or TG61 (B) labeled with ALP and recombinant thyroglobulin. The capture antigen was biotinylated and detection antibodies were labeled with alkaline phosphatase. Serial dilutions of pooled serum samples with thyroglobulin autoantibodies level measured with the Roche Elecsys Anti-Tg assay were used as a calibrator. The luminescent signal is expressed as a ratio of RLU in a blank sample to RLU in an analyzed sample.

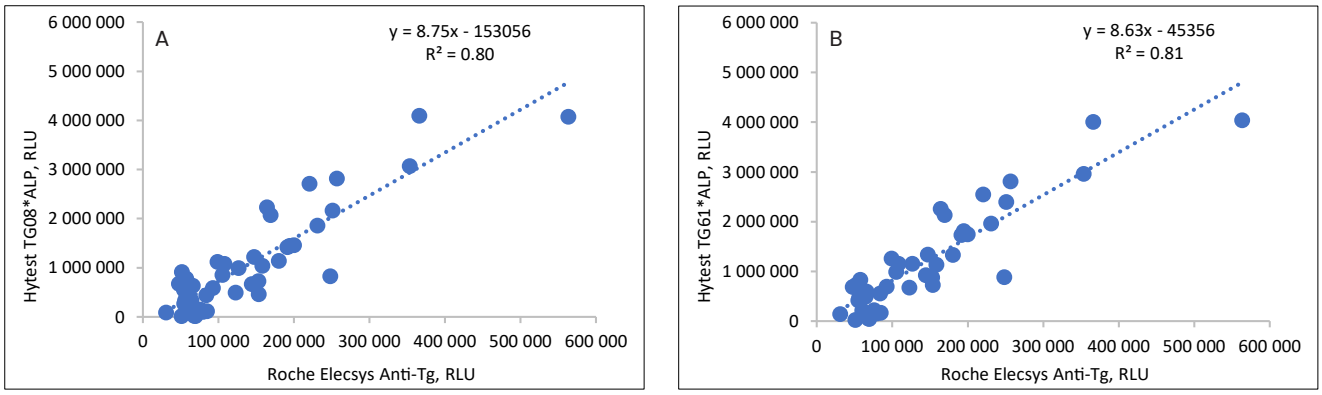

Figure 7.
Scatter plot of thyroglobulin autoantibodies levels measured in 45 serum samples with two sequential competitive assays based on the MAbs TG08 (A) or TG61 (B) and recombinant thyroglobulin in comparison with the Roche Elecsys Anti-Tg assay. The capture antigen was biotinylated and detection antibodies were labeled with alkaline phosphatase. The luminescent signal is expressed in RLU.

REFERENCES

1. **Coscia F, Taler-Vercic A, et al.** The structure of human thyroglobulin. Nature. 2020 Feb; 578(7796):627-30. PubMed PMID: 32025030. PubMed Central PMCID: 7170718.
2. **Spencer CA, Lopresti JS.** Measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer. Nature Clinical Practice Endocrinology & Metabolism. 2008 Apr; 4(4):223-33. PubMed PMID: 18268520.
3. **Clark P, Franklyn J.** Can we interpret serum thyroglobulin results? Annals of Clinical Biochemistry. 2012 Jul; 49 (Pt 4):313-22. PubMed PMID: 22589360.
4. **Frohlich E, Wahl R.** Thyroid Autoimmunity: Role of Anti-thyroid Antibodies in Thyroid and Extra-Thyroidal Diseases. Frontiers in Immunology. 2017; 8:521. PubMed PMID: 28536577. PubMed Central PMCID: 5422478.
5. **Spencer CA, Takeuchi M, et al.** Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. The Journal of Clinical Endocrinology and Metabolism. 1998 Apr; 83(4):1121-7. PubMed PMID: 9543128.
6. **Antonelli A, Ferrari SM, et al.** Autoimmune thyroid disorders. Autoimmunity Reviews. 2015 Feb; 14(2):174-80. PubMed PMID: 25461470.
7. **Erali M, Bigelow RB, et al.** ELISA for thyroglobulin in serum: recovery studies to evaluate autoantibody interference and reliability of thyroglobulin values. Clinical Chemistry. 1996 May; 42(5):766-70. PubMed PMID: 8653905.
8. **Kitamura Y, Narita S, et al.** A Novel Thyroglobulin Immunoassay Using the Specimen-Pretreatment Process Improves the Accuracy of Thyroglobulin Measurements in Anti-Thyroglobulin Positive Specimens. The Journal of Applied Laboratory Medicine. 2021 Nov 1; 6(6):1463-75. PubMed PMID: 34580727.

ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Thyroglobulin	2TG12cc	5E6cc	IgG2b	*In vitro*, EIA
		5F9cc	IgG2a	*In vitro*, EIA. IHC
		TG08	IgG1	*In vitro*, EIA
		TG12	IgG	EIA, recombinant rabbit antibody
		TG14	IgG1	*In vitro*, EIA
		TG16	IgG1	*In vitro*, EIA
		TG23	IgG1	*In vitro*, EIA
		TG33	IgG2b	*In vitro*, EIA
		TG36	IgG1	*In vitro*, EIA
		TG37	IgG1	*In vitro*, EIA
		TG46	IgG2a	*In vitro*, EIA
		TG47	IgG1	*In vitro*, EIA
		TG51	IgG1	EIA, recombinant chimeric antibody
		TG61	IgG1	EIA, recombinant chimeric antibody
		TG64	IgG1	EIA, recombinant chimeric antibody
		TG66	IgG1	EIA, recombinant chimeric antibody

ANTIGENS

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

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