DRG® Thyroglobulin (Tg) (EIA-4126)

CE IVD

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Intended Use: The DRG Thyroglobulin Microplate Elisa test is intended to be used for the quantitative determination of Thyroglobulin levels in human serum. The test is for in vitro diagnostic use only.

SUMMARY AND EXPLANATION OF THE TEST

Human *thyroglobulin (Tg)* is a large glycoprotein (680 kD) that is stored in the follicular colloid of the thyroid gland. It functions as a prohormone in the intrathyroid synthesis of primary thyroid hormones like *Triiodothyronine (T3) and Thyroxine (T4).*

Tg is elevated in thyroid follicular and papillary carcinoma, thyroid adenoma, subacute thyroiditis, Hashimoto's thyroiditis and Graves Disease. Tg levels are found to be normal in patients with medullary thyroid carcinoma. Serial measurements of Tg is most useful in detecting recurrence of differentiated thyroid carcinoma following surgical resection or radioactive iodine ablation. Tg determination is used as an adjunct to iodine scanning but not as a replacement for it. Assessment of Tg levels aids in management of infants with congenital hypothyroidism.

Tg determination has been done with various methods using direct competitive binding R1A and double antibody sandwich IRMA or Elisa, of which latter is more useful. All these methods suffer from interference by endogenous autoantibodies to Tg. It is useful to determine the effect of autoantibodies before screening such patients for levels of Tg. DRG International provides Tg autoanibody Elisa to rule out such interference.

PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal Thyroglobulin antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. Simultaneously the biotin attached to the antibody binds to the streptavidin coated on the microwells resulting in immobilization of the complex. The interaction is illustrated by the following equation.

$$Ag_{(Tg)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ag_{(Tg)} - {}^{Btn}Ab_{(m)}$$

${}^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)

$Ag_{(Tg)}$ = Native Antigen (Variable Quantity)

$Ag_{(Tg)} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibody complex (Variable Quan.))

k_a = Rate Constant of Association

k_{-a} = Rate Constant of Disassociation

$Ag_{(Tg)} - {}^{Btn}Ab_{(m)}$ + $\underline{Streptavidin_{C.W.}} \Rightarrow \underline{immobilized\ complex}$ (IC)

$\underline{Streptavidin_{C.W.}}$ = Streptavidin immobilized on well

Immobilized complex (IC) = Ag-Ab bound to the well

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

$$(IC) + {}^{Enz}Ab_{(Tg)} \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(Tg)} - IC$$

${}^{Enz}Ab_{(Tg)}$ = Enzyme labeled Antibody (Excess Quantity)

${}^{Enz}Ab_{(Tg)} - IC$ = Antigen-Antibodies Complex

k_b = Rate Constant of Association

k_{-b} = Rate Constant of Dissociation

REAGENTS AND MATERIALS PROVIDED.

A. Thyroglobulin Calibrators -1.0 ml/vial - Icons A - F
Six (6) vials of references for Thyroglobulin antigen at levels of **0(A), 2.0 (B), 10.0(C), 40(D), 100(E), and 250(F) ng/ml.** A preservative has been added.
Note: There is no known, internationally accepted thyroglobulin standard available. The Tg used in the serum based calibrators is a highly purified (98+% pure) human Tg preparation that is calibrated gravimetrically against the reference material obtained from Community Bureau of Reference # CRM 457.

B. x-Tg Biotin Reagent -13ml/vial – Icon
One (1) vial containing biotinylated anti-Tg monoclonal mouse IgG in buffer, dye, and preservative.
Store at 2-8°C.

C. Tg Enzyme Reagent -13 ml/vial - Icon
One (1) vial containing anti-thyroglobulin IgG labeled with horseradish peroxidase (HRP) in buffer, dye, and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate- 96 wells - Icon
One 96-well (breakwell modules) microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C

E. Wash Solution - 20 ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.

F. Substrate A -7ml/vlal - Icon S^A
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C

G. Substrate B - 7ml/vial - Icon S^B
One (1) bottle containing hydrogen peroxide (H_20_2) in buffer. Store at 2-8°C.

H. Stop Solution - 8ml**/vial -** Icon
One (1) bottle of stop solution containing a strong acid (1NHCI).
Store at 2-8°C.

I. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date

Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C.

Required But Not Provided:

1. Pipette(s) capable of delivering 50µl and 100 µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5% (optional).

3.Microplate washer or a squeeze bottle (optional).
4.Microplate Reader with 450nm and 620nm wavelength absorbance capability (The 620nm filter is optional).
5.Absorbent Paper for blotting the microplate wells.
6.Plastic wrap or microplate cover for incubation steps.
7.Vacuum aspirator (optional) for wash steps.
8.Timer.
9.Storage container for storage of wash buffer.

10.Distilled or deionized water.
11.Quality Control Materials.

PRECAUTIONS

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red-top venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the specimen is required.

REAGENT PREPARATION:

1.Wash Buffer

Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature (20-27°C) for up to 60 days.

2.Working Substrate Solution

Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B' Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note: Do not use if the working substrate looks blue.

TEST PROCEDURE

Before proceeding with the assay, bring ail reagents, serum references and controls to room temperature (20-27° C).

1. Format the microplate wells for each calibrator, control and patient sample to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50 µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100 µl) of the x-Tg Biotin Reagent to each well. **It is very important to dispense all reagents close to the bottom of the microwell.**
4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap or microplate cover.
5. Incubate for 16-20 hours at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 350 µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can** be **used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container (Avoiding air bubbles). Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of Tg Enzyme Reagent to all wells.

DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION

9. Cover with a plastic wrap. Incubate at room temperature for 120 minutes.
10. Repeat steps 6 & 7.
11. Add 0.100 ml (100 µl) of working substrate to all wells ***(See Reagent Preparation Section).*** **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

12. Cover with a plastic wrap or microplate cover. Incubate at room temperature for 15 minutes.
13. Add 0.050ml (50 µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

ALTERNATIVE SHORT PROCEDURE:

This procedure can be used with the help of a laboratory hematology shaker.

1. Format the microplate wells for each calibrator, control and patient sample to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) of the biotin labeled monoclonal antibody to each well. **It is very important to dispense all reagents close to the bottom of the microwell.**
4. Incubate **at** room temperature for 2 hours white shaking constantly on a hematology shaker at 150 RPM.
5. Follow steps 6-14 as described in the 'Test Procedure' above.

CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of human thyroglobulin (Tg) **in unknown specimens.**

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding Tg concentration in ng/ml on linear graph paper

(do not average the duplicates of the serum references before plotting).

3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of Tg for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.916) intersects the dose response curve at 31.4 ng/ml Tg concentration (See Figure 1).

Note: Computer data reduction software designed for IEMA (ELISA) assays may also be used for the data reduction.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.031	0.027	0
	B1	0.024		
Cal B	C1	0.103	0.101	2
	D1	0.098		
Cal C	E1	0.309	0.328	10
	F1	0.348		
Cal D	G1	1.269	1.157	40
	H1	1.044		
Cal E	A2	2.230	2.179	100
	B2	2.127		
Cal F	C2	2.826	2.857	250
	D2	2.888		
Cont 1	E2	0.069	0.066	1.04
	F2	0.063		
Cont 2	G2	0.401	0.405	12.8
	H2	0.435		
Patient 1	A3	0.950	0.916	31.4
	B3	0.883		

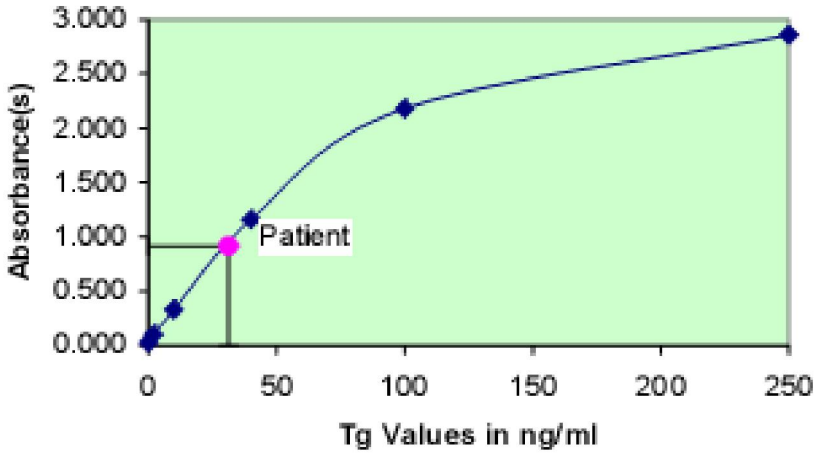
*The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

Q. C. PARAMETERS:

In order for the assay results to be considered valid the following criteria should be met.

1. The absorbance (OD) of calibrator A should be ≤0.10.
2. The absorbance (OD) of calibrator F' should be ≥ 1.3
3. Four out of six quality control pools should be within the established ranges.

Figure 1



RISK ANALYSIS

A. Assay Performance

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient samples with thyroglobulin concentrations above 250 ng/ml may be diluted with the zero calibrator and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from these instructions may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. pipettes, readers, washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis: as required by CE Mark IVD Directive 98/79/EC – for this and other devices, made by DRG, can be requested via e-mail: corp@drg-international.com

Interpretation

1. Laboratory results alone are only one aspect for determining patient care, and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
2. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
3. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted. DRG shall have no liability.
4. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

EXPECTED VALUES

Based on the clinical data gathered by DRG International in concordance with the published literature a normal range of 5-40 ng/ml was established.

Tg is found to be elevated in patients with thyroid follicular and papillary carcinoma, thyroid adenoma, subacute thyroiditis, Hashimoto's thyroiditis and Graves' disease. Low levels of Tg are an indication of thyrotoxicosis factitia.

It is important to keep in mind that any normal range establishment is dependent upon a multiplicity of factors like the specificity of the method, the locale, the population tested and the precision of the method in the hands of technicians. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the technicians using the method with a population indigenous to the area in which the laboratory is located.

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

PERFORMANCE CHARACTERISTICS

A. Precision

The within and between assay precisions of the DRG Thyroglobulin ELISA test systems were determined by analyses on three different levels of pool control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Pool 1	20	11.6	0.93	8.0%
Pool 2	20	39.8	1.96	4.9%
Pool 3	20	117.8	6.00	5.1%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Pool 1	10	10.8	1.05	9.7%
Pool 2	10	41.2	2.22	5.4%
Pool 3	10	114.7	8.20	7.1%

*As measured in ten experiments in duplicate over seven days.

B. Accuracy

The Tg DRG International™ ELISA test system was compared with a reference coated tube radioimmunoassay (IRMA) assay. Biological specimens from population (symptomatic and asymptomatic) were used. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	13.6	y = 2.55 + 0.908 (x)	0.975
Reference	11.4		

The least square regression equation and correlation coefficient indicates excellent method agreement.

C. Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.25 ng/ml

D. Specificity

The cross-reactivity of the DRG Thyroglobulin ELISA method to selected substances was evaluated by adding the interfering substance(s) to a serum matrix at the following concentrations). The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Thyroglobulin needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Thyroglobulin	100 ng/ml	100.0%
Triiodothyronine	1000 ng/dl	N/D
Thyroxine	1000 ng/ml	N/D.
TBG	100 ng/ml	N/D

E. High Dose Effect

Since the assay is sequential in design, high concentrations of Tg do not show the hook effect. Samples with concentrations over 50,000 ng/ml demonstrated extremely high levels of absorbance.

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