Monobind Inc.
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AccuLite
CLIA Microwells

Thyroglobulin (Tg)Test System
Product Code: 2275-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyroglobulin Concentration in Human Serum by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Human thyroglobulin (Tg) is a large glycoprotein (660 kD) that is stored in the follicular colloid of the thyroid gland. It functions as a prohormone in the intra thyroid synthesis of primary thyroid hormones 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 are 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 RIA and double antibody sandwich IRMA or Elisa, of which the 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. *Monobind* provides Tg autoantibody Elisa to rule out such interference. ***(Please see Anti-Tg Acculite® ELISA Product Code: 1075-300).***

3.0 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. 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 Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$Ag_{Tg)} - {}^{Btn}Ab_{(m)}$ + $\underline{Streptavidin}_{CW} \Rightarrow \underline{Immobilized\ complex}$ (IC)
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
$\underline{Immobilized\ complex\ (IC)}$ = 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 light measurable with the use of a microplate luminometer. The enzyme activity on the well is directly proportional to the serum 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.

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

$^{Enz}Ab_{(x\text{-}Tg)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(x\text{-}Tg)}$ – IC = Antigen-Antibodies Complex
k_b = Rate Constant of Association
k_{-b} = Rate Constant of Dissociation

4.0 REAGENTS

Materials Provided:

A. TG Calibrators – 1.0 ml/vial - Icons A – F
Six (6) vials of references for Tg antigen manufactured 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 antigen 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 biotin labeled affinity purified x-Tg monoclonal mouse IgG in buffer, green dye, and preservative. Store at 2-8°C.

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

D. Light Reaction Wells - 96 wells - Icon ⇓
One 96-well white microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Signal Reagent A – 7ml/vial - Icon C[A]
One (1) vial containing luminol in buffer. Store at 2-8°C (see Reagent Preparation Section).

G. Signal Reagent B – 7ml/vial - Icon C[B]
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C (see Reagent Preparation Section).

H. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Luminometer.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplates cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
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

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 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.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

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 use of contaminated devicesAvoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated ranges 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 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.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at room temperature 2-30°C.
2. **Working Signal Reagent Solution -** Store at 2 - 8°C.
   Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE (Time 4hr 05min)

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

*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' 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 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 90 minutes 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 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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 four (4) additional times.**
8. Add 0.100 ml (100µl) of Tg Tracer Reagent to all wells
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Cover with a plastic wrap. Incubate at room temperature for 90 minutes.
10. Repeat steps 6 & 7.
11. Add 0.100 ml (100µl) of working signal reagent to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time.**
    DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION
12. Incubate at room temperature for five (5) minutes in the dark.
13. Read the Relative Light Units (RLU) in each well for 0.5 – 1.0 seconds. **The results should be read within thirty (30) minutes.**

9.1 ALTERNATE PROCEDURE (Time 2hr 05min)

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

1. Format the microplates' 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 and swirl to mix.**
4. Incubate at room temperature for 1 hour while shaking constantly on a hematology shaker at 150 RPM.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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 four (4) additional times.**
7. Add 0.100 ml (100µl) of Tg Tracer Reagent to all wells
8. Incubate at room temperature for 1 hour while shaking constantly on a hematology shaker at 150 RPM.
9. Repeat steps 6-7 as described in the 'Test Procedure' above.
10. Follow steps 11-13 to develop signal and measure.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Tg in unknown specimens.
1. Record the RLUs (Relative Light Units) obtained from the printout of the luminometer as outlined in Example 1.
2. Plot the RLUs for each duplicate serum reference versus the corresponding Thyroglobulin concentration in ng/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.

4. To determine the concentration of Tg for an unknown, locate the average RLUs 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 RLUs (17462) of the unknown intersects the calibration curve at 51.9 ng/ml Tg concentration (See Figure 1)*.

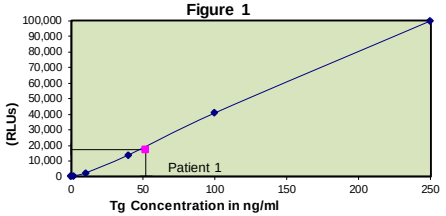
Note: Computer data reduction software designed for CLIA assays may also be used for the data reduction. **If such software is utilized, the validation of the software should be ascertained.**

EXAMPLE 1

Sample ID	Well Position	RLUs	Mean RLUs	Value (ng/ml)
Cal A	A1	65	68	0
	B1	71		
Cal B	C1	410	448	2
	D1	487		
Cal C	E1	2727	2425	10
	F1	2122		
Cal D	G1	11503	12325	40
	H1	13147		
Cal E	A2	41436	40936	100
	B2	40436		
Cal F	C2	101799	100000	250
	D2	98201		
Patient 1	E2	17605	17462	***51.9***
	F2	17319		

* 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. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the 'F' calibrator (greatest light output). This conversion eliminates differences cause by efficiency of the various instruments that can be used to measure light output.

Figure 1



11.0 Q.C. PARAMETERS

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

1. The Dose Response Curve (80%; 50% & 20% intercepts) should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve 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. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
9. 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.
10. 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.
11. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. 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.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. 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, Monobind shall have no liability.
6. 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.
7. 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.

13.0 EXPECTED VALUES

Based on the clinical data gathered by Monobind in concordance with the published literature a normal range was established.

Table 1: Expected Values for TG

POPULATION	RANGE
Adult	3.5 – 56 ng/ml

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.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Tg AccuLite® CLIA test system were determined by analyses on three different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation 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.
A	20	5.6	0.5	8.9%
B	20	15.0	1.10	7.3%
C	20	65.2	6.00	9.2%

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

Sample	N	X	σ	C.V.
A	10	6.1	0.8	13.1%
B	10	15.5	1.2	7.7%
C	10	63.2	6.1	9.7%

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

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 µIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.031 ng/ml

14.3 Accuracy

The Tg AccuLite® CLIA test system was compared with a reference method 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
Monobind	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.

14.4 Specificity

The cross reactivity of the Thyroglobulin Chemiluminescence method to selected substances was evaluated by adding the interfering substance(s) to a serum matrix at the following concentration(s). 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	Concentration	Cross Reactivity
Thyroglobulin	100 ng/ml	100.0%
Triiodothyronine	1000 ng/dl	N/D
Thyroxine	1000 ng/ml	N/D
TBG	100 ng/ml	N/D

14.5 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 intensity of light emission.

15.0 REFERENCES

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9. Spencer CA, LoPresti JS, Fatemi S, Nicoloff JT, "Detection of residual and recurrent differentiated thyroid carcinoma by serum thyroglobulin measurements", *Thyroid*, 9, 435-41 (1999).
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MP2275 **Product Code: 2275-*300***

Size		96(A)
Reagent (fill)	A)	1ml set
	B)	1 (13ml)
	C)	1 (13ml)
	D)	1 plate
	E)	1 (20ml)
	F)	1 (7ml)
	G)	1 (7ml)

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IVD 2° 8° CE
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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity