Thyroglobulin (Tg) Test System
Product Code: 2225-300

1.0 INTRODUCTION

Usable: Monobind’s Thyroglobulin Microplate Elisa test is intended for the quantitative determination of thyroglobulin (Tg) levels in human serum. The test is for in vitro diagnostic use only.

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 intrathyroid synthesis of primary thyroid hormones like Triiodothyronine (T3) and Thyroxine (T4).

Tg is elevated in thyroid follicular and papillary carcinoma, thyroid adenoma, subacute thyroitis, Hashimoto’s thyroids and Graves Disease. Tg levels are found to be normal in patients with medullary thyroid carcinoma. A high Tg is most useful in detecting recurrence of differentiated thyroid carcinoma following surgical resection or radioactive iodine ablation.

Tg determination has been done with various methods using direct competitive binding RIA 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 autoantibodies before screening such patients for levels of Tg. Monobind provides Tg autoantibody Elisa to rule out such interference. (Please see Monobind Anti Tg Elisa Cat# 1025-300).

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 microwell plate through the interaction of streptavidin coated on the well and extensively added biotinylated monoclonal Thyroglobulin antibody.

Monobind’s biotin antibody binds to the streptavidin coated on the microwells resulting in immobilization of the complex. The interaction is illustrated by the following equation:

\[ \text{Ag}(\text{Tg}) + \text{BtnAb(m)} \]

**4.0 REAGENTS**

Materials Provided:

A. Thyroglobulin Calibrators – 1.0 ml/vial - Icons A - F

Six (6) vials of references for Thyroglobulin antigen (Tg) at levels of B(A), B(2), B(5), B(10), B(40), B(100), and B(250) mg/dl. A preservative has been added.

Note: There is no known, internationally accepted thyroid globulin 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 maintained from Community Bureau of Reference # CRM 457.

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

C. Tg Enzyme Reagent – 13ml/vial - Icon H
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 J
One (1) vial (96 well glass 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 - Icon K
One (1) vial contains surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate Reagent – 12ml/vial - Icon L
One (1) bottle contains tetramethylbenzidine (TMB) and hydrogen peroxide (H2O2) in Buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon M
One (1) bottle contains a strong acid (H2SO4). Store at 2-30°C.

H. Product Instructions

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

Note 2: Avoid exposure to light and heat. Opened reagents are stable for sixty (60) days when stored at 2-8°C.

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Pipette(s) capable of delivering 50µl and 100µl volumes with a precision of better than 1.5%.

2. Dispensers(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 and 620nm wavelength absorbance capability (The 620nm filter is optional)

5. Absorbent Paper for blotting the microplate wells.

6. Plastic cover for microplates.

7. Vacuum aspirator (optional) for wash steps.

8. Timer.

9. Storage container for storage of wash buffer.

10. Distilled or deionized water.


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 minimally 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 urine, and the usual precautions in the collection of venipuncture samples should be observed. Serum should be obtained to establish normal values. A fasting morning serum sample should be obtained. The blood should be collected in a plain red-top vial without additives or gel. 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 <2°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the specimen is required.

7.0 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 tested. 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.

8.0 REAGENT PREPARATION

1. Wash Buffer

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

Note 1: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE (Time 4hr 15min)

**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.

4. It is very important to dispense all reagents close to the bottom of the microplate.

5. Swirl the plate gently for 20-30 seconds to mix. Cover with a plastic wrap or microplate cover.

6. Incubate at room temperature for 1 hour while shaking.

7. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

8. Add 3550µl 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 bottom by squeezing the cap (Avoiding air bubbles). Discard the wash and repeat two more times.

9. Add 0.100 ml (100µl) of Tg Enzyme Reagent to all wells

DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION

10. Incubate with a plastic wrap. Incubate at room temperature for 120 minutes.

11. Repeat steps 8 & 7.

12. Add 0.100 ml (100µl) of substrate to all wells. Always add reagents in the same order in to minimize reaction time differences between wells.

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

13. Add 0.1050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. Always add reagents in the same order.

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.

9.1 ALTERNATE PROCEDURE (Time 2hr 15min)

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 0.100 ml (100µl) 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 hematocrit shaker at 150 RPM.

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

6. Add 35µl 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 bottom by squeezing the cap (Avoiding air bubbles). Discard the wash and repeat two more times.

7. Add 0.100 ml (100µl) of Tg Enzyme Reagent to all wells

DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION

8. Incubate at room temperature for 1 hour while shaking constantly on a hematocrit shaker at 150 RPM.

9. Repeat steps 5-6 of the ‘Test Procedure’ above.

10. Follow steps 11-14 to develop color and measure.
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 ordinate of the absorbance values and find the intersecting point on the graph, and read the concentration in (ng/ml) from the ordinate axis of the graph (The duplicates of the unknown sera should be averaged as indicated).
3. Draw the best-fit curve through the plotted points.

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. The intersecting point on the graph, and read the concentration in (ng/ml) from the ordinate axis of the graph (The duplicates of the unknown sera should be averaged as indicated). In the following example, the average absorbance (0424) intersects the dose response curve at 25.2 ng/ml Tg concentration (See 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 absorbance (OD) of calibrator A should be ≤ 0.10.
2. The absorbance (OD) of calibrator F should be ≥ 1.3.
3. Four out of five quality control pools should lie within the established range.

12.0 RISK ANALYSIS

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

12.1 Assay Performance

1. It is important to follow some of the reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) mm to avoid dilution of the sample.
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 substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop 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 assay and/or decantation on each 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.
10. 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.
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 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 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 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. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could alter the reaction or cause false test results. The final batches are incorrectly identified, Monobind shall have no liability.
5. 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.

13.0 EXPECTED VALUES

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

<table>
<thead>
<tr>
<th>Tg Values in ng/ml</th>
<th>POPULATION RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>3.5 – 56 ng/ml</td>
</tr>
</tbody>
</table>

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.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The Tg AccuBind™ 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.

14.2 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 concentration. The assay sensitivity was found to be 0.64 ng/ml.

14.3 Accuracy

The analytical 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 concentration. The assay sensitivity was found to be 0.64 ng/ml.

14.4 Specificity

The cross-reactivity of the Thyroglobulin AccuBind™ ELISA 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 adding a known amount of the interfering substance to dose of Thyroglobulin needed to produce the same absorbance.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>Cross Reactivity</th>
<th>Method Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroglobulin</td>
<td>1000 ng/ml</td>
<td>100.0%</td>
<td>2σ = 0.64 ng/ml</td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>1000 ng/dl</td>
<td>N/D</td>
<td></td>
</tr>
<tr>
<td>Thyroxine</td>
<td>1000 ng/ml</td>
<td>N/D</td>
<td></td>
</tr>
<tr>
<td>Tg</td>
<td>100 ng/ml</td>
<td>N/D</td>
<td></td>
</tr>
</tbody>
</table>

15.0 REFERENCES


Revision: 3
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DCC: 0704
Cat #: 2225-300

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