The essential reagents required for an immunoenzymometric assay include:

- **Monobind's Anti-Tg Acculite™** Cat# 1075-300).
- A reagent to rule out such interference.

1. INTRODUCTION

1.0 INTRODUCTION

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 thyroids, Hashimoto’s thyroids and Graves Disease. Tg levels are found to be normal in patients with medullary thyroid carcinoma. Measurement 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 diagnostic tool to detect the presence of thyroid carcinoma. Tg is elevated in thyroid follicular and papillary carcinoma, thyroid adenoma, subacute thyroids, Hashimoto’s thyroids and Graves Disease. Tg levels are found to be normal in patients with medullary thyroid carcinoma. Measurement 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 diagnostic tool to detect the presence of thyroid carcinoma.

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

9.0 TEST PROCEDURE (Time 4hr 05min)

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 22°C).

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

1. Format the microplates’ wells for each calibrator, control and sample into the assigned wells.
2. Pipette 0.050 ml (50µl) of the appropriate calibrator, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) of the x-Tg Biotin Reagent to each well.
4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap or microplate cover.
5. Incubate for 50 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 350µl of wash buffer (see Reagent Preparation Section), developing and clearing the plate. Allow the wash bottle to rest on the plate.
8. Cover with a plastic wrap. Incubate at room temperature for 90 minutes.
9. Note: Always make the wash buffer to room temperature.
10. Follow steps 11-13 to develop signal and measure.

9.1 ALTERNATE PROCEDURE (Time 2hr 05min)

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

1. Format the microplates’ wells for each calibrator, control and patient sample to be assayed in duplicate. Replace any unused microcell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.050 ml (50µl) of the appropriate calibrator, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) of the x-Tg Biotin Reagent to each well.
4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap or microplate cover.
5. Incubate at room temperature for 90 minutes.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), developing and clearing the plate. Allow the wash bottle to rest on the plate.
8. Cover with a plastic wrap. Incubate at room temperature for 90 minutes.
9. Note: Always make the wash buffer to room temperature.
10. Follow steps 11-13 to develop signal and measure.

1.0 INTRODUCTION

Monobind’s Thyroglobulin Acculite™ Chemiluminescence 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.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Human thyroglobulin is a large glycoprotein (660 kD) that is stored in the follicular colloid of the thyroid gland. It functions as a prohormone in the intrathyroidal synthesis of primary thyroid hormones triiodothyronine (T3) and thyroxine (T4). Thyroglobulin is used as an adjunct to iodine scanning but not as a diagnostic tool to detect the presence of thyroid carcinoma.

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. “Biosecurity in Microbiological and Biomedical Laboratories,” 2nd Edition, 1988, HHS

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

5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

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, it is recommended that the specimen be centrifuged. 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 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 ranges for monitoring assay performance. These controls should be treated as unknowns and values determined in the same manner as those of routine samples. Quality control trends 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 deterioration of reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer
2. Working Standard Solution - Store at 2 - 8°C
3. Determine the amount of reagent needed and prepare fresh reagents. Fresh reagents should be used to determine the reason for the variations.

9.0 TEST PROCEDURE (Time 4hr 05min)

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 22°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 microcell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.050 ml (50µl) of the appropriate calibrator, 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 and stir to mix.
4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap or microplate cover.
5. Incubate for 50 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 350µl of wash buffer (see Reagent Preparation Section), developing and clearing the plate. Allow the wash bottle to rest on the plate.
8. Cover with a plastic wrap. Incubate at room temperature for 90 minutes.
9. Note: Always make the wash buffer to room temperature.
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 RLU’s (Relative Light Units) obtained from the printout of the luminometer as outlined in Example 1.

2. Plot the RLU’s for each duplicate serum reference versus the corresponding concentration in ng/ml on a 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 RLU’s 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 RLU’s (17462) of the unknown intersects the calibration curve at 63.2 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.

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 is available on request from Monobind Inc.

12.1 Assay Performance

It is important that the time of reaction in each well is held constant to achieve reproducible results.

1. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.

2. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.

3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.

4. 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.

5. Failure to remove adventitious solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurrious results.

6. Use components from the same lot. No intermixing of reagents from different batches.

7. 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.

8. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure proper collection and proper device usage.

9. 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.

10. 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 are only an aid 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, then the assay results are generally invalid.

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.

6. Patient samples with Thyroglobulin concentrations above 250 ng/ml may be used in lieu of a dose response curve.

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

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroglobulin</td>
<td>100 ng/ml</td>
<td>0.00%</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>100 ng/ml</td>
<td>N/D</td>
</tr>
<tr>
<td>Thyroglobulin</td>
<td>1000 ng/ml</td>
<td>N/D</td>
</tr>
<tr>
<td>Thyrogen</td>
<td>1000 ng/ml</td>
<td>N/D</td>
</tr>
<tr>
<td>T3</td>
<td>100 ng/ml</td>
<td>N/D</td>
</tr>
</tbody>
</table>

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 Thyroglobulin AccuLite™ CLIA microplate assay 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)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>15</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3: Between Assay Precision (Values in ng/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

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www.monobind.com

* 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 RLU’s of the calibrators have been normalized to 100,000 RLU’s for the ‘F’ calibrator (greatest light output). This conversion eliminates differences caused by efficiency of the various instruments that can be used to measure light output.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the value at the 0.10 µIU/ml calibrator and using the 2x (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.031 ng/ml.

14.3 Accuracy

The Thyroglobulin AccuLite™ CLIA procedure was compared with a reference thyroid chemiluminescence (IRMA) assay. Biological specimens from population (symptomatic and asymptomatic) were used. The data obtained is displayed in Table 4.

Table 4: Least Square Regression Analysis

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroglobulin</td>
<td>100 ng/ml</td>
<td>0.975</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>1000 ng/ml</td>
<td>0.975</td>
</tr>
<tr>
<td>T3</td>
<td>100 ng/ml</td>
<td>0.975</td>
</tr>
</tbody>
</table>

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 concentrations(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.

Table 5: Cross Reactivity of Thyroglobulin Chemiluminescence

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroglobulin</td>
<td>100 ng/ml</td>
<td>100.0%</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>1000 ng/ml</td>
<td>N/D</td>
</tr>
<tr>
<td>T3</td>
<td>1000 ng/ml</td>
<td>N/D</td>
</tr>
<tr>
<td>T4</td>
<td>1000 ng/ml</td>
<td>N/D</td>
</tr>
</tbody>
</table>

14.5 High Dose Effect

Since the assay is sequential in design, high concentrations of Tg do not show the blank effect. Samples with concentrations over 50,000 ng/ml demonstrated extremely high intensity of light emission.

15.0 REFERENCES

3. Mayo Medical Laboratories. 1997Catalog, Rochester, MN.

Revision: 3 Date: 052912 DCO: 0712 Cat #: 2257-200

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