Hormones triiodothyronine (T3) and thyroxine (T4) are stored in the follicular colloid of the thyroid gland. It functions as a reservoir for primary thyroid hormones triiodothyronine (T3) and thyroxine (T4).

**SUMMARY AND EXPLANATION OF THE TEST**

Disease. Tg levels are found to be normal in patients with thyroid adenoma, subacute thyroids, Hashimoto's thyroids and Graves Disease. Tg levels are found to be normal in patients with autoimmune thyroiditis. Tg levels are to be determined in patients with hypothyroidism. Adequate serum 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 vacutainer tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the cells from the serum. 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 fastiging results. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen 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. The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed.

**Materials Provided:**
- One (1) vial containing anti-Thyroglobulin mouse IgG labeled with horseradish peroxidase (HRP) in buffer, dye, and additives or gel barrier. Avoid extended exposure to heat and light.
- One (1) vial containing hydrogen peroxide (H2O2) in buffer.
- One (1) vial containing luminol in buffer. Store at 2-8°C (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 the procedure up to four (4) additional times.
- One (100 ml) (Tg) Tracer Reagent to all wells

DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION

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.

**Calculation of Results**

A dose response curve is used to ascertain the concentration of Tg in unknown specimens. An antibody will have been preincubated with either a range of Tg concentrations or a single concentration.

Assay Kit

1. Format the microwells' wells for each calibrator, control and patient

2. Pipette 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 swifl to mix.

3. Incubate at room temperature for 1 hour while shaking on a hematology shaker at 150 RPM.

4. Discard the contents of the microwell by decantation or aspiration, four (4) additional times for a total of five (5) washes. A manual or automatic 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 the procedure up to four (4) additional times.

5. Add 0.100 ml (100µl) of Tg Tracer Reagent to all wells

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 the procedure up to four (4) additional times.

7. Add 0.300ml (300µl) 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 the procedure up to four (4) additional times.

8. Add 0.100 ml (100µl) of Tg Tracer Reagent to all wells

9. Do not use reagents beyond the kit expiration date.

10. Follow steps 6-7 & 9. Use 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.

11. Do not shake the plate after enzyme addition

**Alternate Procedure (Time 2hr 05min)**

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

1. Format the microwells’ 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. Avoid extended exposure to heat and light. It is very important to dispense all reagents close to the bottom of the microwell and swifl to mix.

4. Incubate at room temperature for 1 hour while shaking on a hematology shaker at 150 RPM.

5. Discard the contents of the microwell by decantation or aspiration, four (4) additional times for a total of five (5) washes. A manual or automatic 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 the procedure up to four (4) additional times.

6. Add 0.300ml (300µl) 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 the procedure up to four (4) additional times.

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

8. 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 the procedure up to four (4) additional times.

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

10. Repeat steps 6-7 & 9. Use 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.

11. Do not shake the plate after enzyme addition

12. Incubate at room temperature for five (5) minutes in the dark.

13. Add 0.100 ml (100µl) of luminol in buffer. If desired, repeat the procedure up to 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 the procedure up to four (4) additional times.

14. Add 1.000 ml (1000µl) of Wash Buffer to each well for 5 minutes. The results should be read within thirty (30) minutes.
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. 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**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Well Position</th>
<th>Mean RLUs</th>
<th>Value (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td>A1</td>
<td>65</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>71</td>
<td>0</td>
</tr>
<tr>
<td>Cal B</td>
<td>C1</td>
<td>410</td>
<td>448</td>
</tr>
<tr>
<td></td>
<td>D1</td>
<td>487</td>
<td>2</td>
</tr>
<tr>
<td>Cal C</td>
<td>E1</td>
<td>2127</td>
<td>2425</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>2122</td>
<td>10</td>
</tr>
<tr>
<td>Cal D</td>
<td>G1</td>
<td>11503</td>
<td>12325</td>
</tr>
<tr>
<td></td>
<td>H1</td>
<td>1322</td>
<td>40</td>
</tr>
<tr>
<td>Cal E</td>
<td>A2</td>
<td>41436</td>
<td>40938</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>40436</td>
<td>100</td>
</tr>
<tr>
<td>Cal F</td>
<td>C2</td>
<td>107199</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>D2</td>
<td>86201</td>
<td>250</td>
</tr>
<tr>
<td>Patient 1</td>
<td>E2</td>
<td>17605</td>
<td>17462</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>17319</td>
<td>51.9</td>
</tr>
</tbody>
</table>

* 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 in light output.

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 maintenance.
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.

**12.0 RISK ANALYSIS**

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

12.1 Assay Performance

1. The Dose Response Curve (80%; 50% & 20% intercepts) and the RLUs of the calibrators be used in lieu of a dose response curve. 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 in light output.

2. Four out of six quality control pools should be within the listed ranges and assay requirements.

3. The reagents for the test system have been formulated to measure light output.

4. Valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

5. If tests by efficient method as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind will not support the assay.

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.

12.2 Interpretation

Measurements and interpretation of results must be performed by a skilled individual or trained professional.

2. Laboratory results alone are one only aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other diagnostics.

3. The reagents for the test system have been formulated to measure light output.

5. If tests by efficient method as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind will not support the assay.

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**

<table>
<thead>
<tr>
<th>POPULATION</th>
<th>RANGE</th>
<th>Tg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adult</strong></td>
<td>3.5 – 56 ng/ml</td>
<td>Tg is found to be elevated in patients with thyroid follicular and papillary carcinomas, thyroid adenoma, subacute thyroiditis, Hashimoto’s thyroiditis and Graves’ disease. Low levels of Tg are an indication of thyrotoxicosis factitia.</td>
</tr>
</tbody>
</table>

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Tg AccuLite® CLIA test system was determined by analyses on three different levels of pool control sera. The numbers, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

**Table 2**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>5.6</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>5.0</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>6.2</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>6.1</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>6.3</td>
</tr>
</tbody>
</table>

*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 2e (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 were evaluated by the cross-reacting 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 and dose of Thyroglobulin needed to produce the same absorbance.

**Table 4**

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean Value (ng/ml)</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monobind</td>
<td>13.6</td>
<td></td>
<td>0.975</td>
</tr>
</tbody>
</table>

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 and dose of Thyroglobulin needed to produce the same absorbance.

**Table 5**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (ng/ml)</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroglobulin</td>
<td>100 ng/ml</td>
<td>100.0%</td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>1000 ng/ml</td>
<td>ND</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>1000 ng/ml</td>
<td>ND</td>
</tr>
<tr>
<td>TBG</td>
<td>100 ng/ml</td>
<td>ND</td>
</tr>
</tbody>
</table>

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