**1.0 INTRODUCTION**

Intended Use: The Quantitative Determination of Thyroid Peroxidase (TPO) antibodies in serum by a Microplate Enzyme Immunoassay, Chemiluminescence.

**2.0 SUMMARY AND EXPLANATION OF THE TEST**

Antibodies to thyroid peroxidase have been shown to be characteristic from patients with Hashimoto thyroiditis (95%), idiopathic myasthenia (90%) and Graves Disease (80%). In fact, 72% of patients with Hashimoto thyroiditis have at least some degree of thyroid dysfunction. This has led to clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction.

Measurements of antibodies to TPO have in the past been done by Passive Hemagglutination (PHA). PHA tests do not have the sensitivity of chemiluminescence immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of CLA (up to 10(5) of subclinical levels of antibodies to TPO. In addition, the results are quantitated by a luminometer, which eliminates subjective interpretation.

**3.0 CLA MICROWELLS**

Monobind’s microplate chemiluminescence immunoassay methodology provides high sensitivity with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted patient specimen, or control is first added to a microplate well. Biotinylated Thyroid Peroxidase Antigen (TPO) is added, and the biotin conjugates. Reaction between the autoantibodies to TPO and the biotinylated TPO to form an immune complex, which forms:

\[ h-Ab(X-TPO) \rightarrow BtnAg(TPO) \]

Repeatedly, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated below:

\[ h-Ab(X-TPO) \rightarrow BtnAg(TPO) = Immune Complex (Variable Quantity) \]

**4.0 MATERIALS**

**Materials Provided:**
A. Anti-TPO Calibrators – 1.0 ml - Vials A-F
B. TPO Biotin Conjugate – 1ml - Vials
C. Anti-TPO Tracer Reagent – 1ml - Vials
D. Light Reaction Wells – 96 wells – Vials
E. Serum Diluent Concentrate – 20ml/vial
F. Wash Buffer
G. Signal Reagent A – 7ml - Vials
H. Signal Reagent B – 7ml - Vials
I. Product Insert

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 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 0.010ml (10μl) and 0.025ml (25μl) volumes with a precision of ±0.0.5%.
2. Dispenser(s) for direct addition if not used within 36 hours after mixing biotinylated antigen and a serum containing the auto-Ab complex in a second incubation is separated from unreacted components by aspiration and/or decantation. The unbound components are then added to the microwells. This conjugates binds to the immune complex, which forms:

\[ h-Ab(X-IgG) \rightarrow BtnAg(TPO) \rightarrow Imnrdzld Antigen (IC) \]

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity, determined by reaction with a substrate that generates light, in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several reference calibrators of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained.

**5.0 PRECAUTIONS**

**For In Vitro Diagnostic Use**

Not for Internal or External Use in Humans or Animals

All Monobind 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 testing. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous. (See MRC. 1980. Manual of Laboratory and General Practice). Storage and handling procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health. “Bioseutary in Microbiological and Biomedical Laboratories.” 2nd Edition, 1988. HHS Publication No. (CDC) 88-8395.

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

**6.0 SPECIMEN COLLECTION AND PREPARATION**

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The specimens shall be blood serum or heparinized plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established performance criteria, raw serum, not an automated or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is used, it should be depressurized by depressing the internal mechanism of the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.

**7.0 TEST PROCEDURE**

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**Procedure:**

1. Assemble the microwell strips for each serum reference calibrator, control and patient specimen to be assayed in duplicate in sequential microwell strips back into an aluminum bag, seal and store at 2-8°C.

2. Pipette 0.025ml (25μl) of the appropriate serum reference calibrator, control or diluted patient specimen into the assigned well.

3. Add 0.100ml (100μg) of TPO Biotinylated Conjugate Solution.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 30 minutes at room temperature.

6. Blot the contents of the microwell by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

7. Add 0.350ml (350μl) of wash buffer (see Reagent Preparation Section) decad (lap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes.

8. Add 0.050ml (50μl) of anti-TPO Tracer Reagent to all wells.

9. Incubate for 30 minutes at room temperature.

10. Discard the contents of the microwells by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

11. Add 0.350ml (350μl) of wash buffer (see Reagent Preparation Section) decad (lap 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, it should be depressurized by depressing the internal mechanism of the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.

12. Add 0.100ml (100μg) of anti-TPO Tracer Reagent to all wells.

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

14. Decant the Artemis positive controls to monitor assay performance. Each laboratory should assay and establish reference intervals for the specimen is required.

15. Negate the concentration of the specimen. **DO NOT SHAKE PLATE AFTER SIGNAL ADDITION**

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

17. Add 0.100ml (100μg) of Light Reaction Wells in each well in a suitable container with or without denatured water. Store at 2-8°C.

18. Wash Buffer

Dilute contents of Wash Concentrate to 1000ml with distilled or denatured water in a suitable storage container. Store diluted buffer at room temperature 2-30°C.

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Dilute contents of Wash Concentrate to 1000ml with distilled or denatured water in a suitable storage container. Store diluted buffer at room temperature 2-30°C.

3. 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. Mix A and B in 1ml of B per two (2) eight well strips (A slight excess of solution is mandatory) and store at room temperature. Store all reagents >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high doses of iodides (≥5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

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

**9.0 TEST PROCEDURE**

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Before proceeding with the assay, bring all reagents, reference calibrators and controls to room temperature (20-25°C).

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

1. Assemble the microwell strips for each serum reference calibrator, control and patient specimen to be assayed in duplicate in sequential microwell strips back into an aluminum bag, seal and store at 2-8°C.

2. Pipette 0.025ml (25μl) of the appropriate serum reference calibrator, control or diluted patient specimen into the assigned well.

3. Add 0.100ml (100μg) of TPO Biotinylated Conjugate Solution.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

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8. Add 0.050ml (50μl) of anti-TPO Tracer Reagent to all wells.

9. Incubate for 30 minutes at room temperature.

10. Discard the contents of the microwells by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

11. Add 0.350ml (350μl) of wash buffer (see Reagent Preparation Section) decad (lap 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, it should be depressurized by depressing the internal mechanism of the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.

12. Add 0.100ml (100μg) of working signal reagent to all wells (see Reagent Preparation Section). Add one or more standards in each well in the same order to minimize reaction time differences between wells.

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

14. Add 0.100ml (100μg) of Light Reaction Wells in each well in a suitable container with or without denatured water. Store at 2-8°C.
10.0 CALCULATION OF RESULTS

A reference curve is used to ascertain the concentration of anti-TPO (x-TPO) in unknown specimens.
1. Record the RLUs obtained from the printout of the microplate luminometer as outlined in Example 1.
2. Plot the mean RLUs for each duplicate serum reference sample for this should not extend beyond ten (10) minutes to avoid assay drift.
3. Plot the mean RLUs for each duplicate serum reference sample for this should not extend beyond ten (10) minutes to avoid assay drift.
4. To determine the concentration of x-TPO for an unknown, locate the average RLUs for each unknown on the vertical axis of the graph, mark the intersecting point, draw a line from the intersecting point on the graph to the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs (8862) of the unknown intersects the concentration curve at (367 U/ml) x-TPO concentration. (See Figure 1).
5. The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

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. Pipette in a fashion such that you do not exceed beyond ten (10) minutes to avoid assay drift.
3. Highly leptmic, hemolyzed or grossly contaminated specimen(s) should not be included.
4. If more than one (1) plate is used for an experiment, it is recommended to repeat the same reaction for those specimens on different plates.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added to the same plate. Any deviation from Monobind’s IFU may result in poor reproducibility.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor reproducibility.
7. Use components from the same lot. No intermixing of reagents is allowed.
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 result in 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. The number (n), mean (x) and standard deviation (σ) of samples are given.

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

Table 1. Values in excess of 40 U/ml are considered positive for the presence of anti-TPO antibodies.

<table>
<thead>
<tr>
<th>Number (n)</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (x)</td>
<td>17.6</td>
</tr>
<tr>
<td>Standard deviation (σ)</td>
<td>10.8</td>
</tr>
<tr>
<td>Upper 95% (+2σ) level</td>
<td>39.2</td>
</tr>
</tbody>
</table>

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of ‘normal persons’, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, a priori should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts 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 Anti-TPO 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within Assay Precision (Values in U/ml)</th>
<th>Between Assay Precision (Values in U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool 1</td>
<td>20</td>
<td>9.1</td>
</tr>
<tr>
<td>Pool 2</td>
<td>20</td>
<td>55.7</td>
</tr>
<tr>
<td>Pool 3</td>
<td>20</td>
<td>121.8</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate.

14.2 Sensitivity
The Anti-TPO AccuLite® CLIA test system has a sensitivity of 1.0 IU/ml. The sensitivity was ascertained by determining the reference (x) values. The Anti-TPO AccuLite® CLIA test system has a sensitivity of 1.0 IU/ml. The sensitivity was ascertained by determining the reference (x) values. The Anti-TPO AccuLite® CLIA test system has a sensitivity of 1.0 IU/ml. The sensitivity was ascertained by determining the reference (x) values.

14.4 Specificity
The Anti-TPO AccuLite® CLIA test system was compared with a reference method. Biological specimens from normal and disease state populations were used. The disease states included Hashimoto’s thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimens was 125. The least square regression equation and the correlation coefficient calculated by determining the similarity of the ‘y’ coefficient and the using the 20 (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy
The Anti-TPO AccuLite® CLIA test system was compared with a reference method. Biological specimens from normal and disease state populations were used. The disease states included Hashimoto’s thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimens was 125. The least square regression equation and the correlation coefficient calculated by determining the similarity of the ‘y’ coefficient and the using the 20 (95% certainty) statistic to calculate the minimum dose.

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14.4 Specificity
Interferences from ANA, DNA, thyroid peroxidase (TPO) and rheumatoid antibodies were found to be insignificant in the assay system.

15.0 REFERENCES

Revision: 4 Date: 2019-JUL-16 DCO: 1353

Glossary of Symbols:
- MP1175 Product Code: 1175-300

For more information, visit our website to learn more about our products and services.