**AgAbC.W.** + **EnzAgAbC.W.**

**ka**

Interaction is illustrated by the equation in the following:

\[ K = \frac{ka}{k-a} = \text{Equilibrium Constant} \]

\[ k-a = \text{Rate Constant of Disassociation} \]

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The activity in the antibody-bound fraction is inversely proportional to the antigen concentration.

Using several different serum reference calibrators of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

### 4.0 REAGENTS

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. T4 Calibrators</td>
<td>1ml/vial – Icons A-F</td>
<td>Store at 2-8°C</td>
</tr>
<tr>
<td>B. T4 Antibody Plate</td>
<td>96 wells – Icon C</td>
<td>Store at 2-8°C</td>
</tr>
<tr>
<td>C. T3/T4 Conjugate Buffer</td>
<td>13 ml/vial – Icon C</td>
<td>Store at 2-8°C</td>
</tr>
<tr>
<td>D. T4 Antibody Coated Plate</td>
<td>96 wells – Icon C</td>
<td>Store at 2-8°C</td>
</tr>
<tr>
<td>E. Substrate B</td>
<td>7ml/vial – Icon SB</td>
<td>Store at 2-8°C</td>
</tr>
<tr>
<td>F. Substrate A</td>
<td>7ml/vial – Icon SA</td>
<td>Store at 2-8°C</td>
</tr>
<tr>
<td>G. Stop Solution</td>
<td>8ml/vial – Icon STOP</td>
<td>Store at 2-8°C</td>
</tr>
</tbody>
</table>

### 3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 5)

The essential components of a solid phase enzyme immunoassay include immobilized antigen, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized antigen, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. The interaction is illustrated by the equation in the following:

\[ E_{\text{Ag}} + \text{Ag} + \text{Ab.C.W.} = \text{Ag-Ab.C.W.} \]

\[ E_{\text{Ag}} + \text{Ab.C.W.} = \text{Ag-Ab.C.W.} \]

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025 and 0.050 ml (25 & 50 µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 and 0.350 ml (100 & 350 µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for substrate preparation.
4. Microplate washer or a squeeze bottle (optional).
5. Vacuum aspirator (optional) for wash steps.
6. Test tubes for preparation of enzyme conjugate.

4.2 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 at the Center for Disease Control and the Biodefense Program of Microbiological and Biomedical Laboratories,” 2nd Edition, 1988, HHS Publication No. (CDC) 88-6395.

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

### 5.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid (see below) ranges to establish performance of the assay. These controls should be treated as unknowns and values determined in every test procedure performed. Quality controls should be employed to follow the performance of the supplied reagents. Pertinent statistical methods should be used to ascertain trends.

The individual laboratory should set up its own control panel for the T4 test. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate uncontrolled conditions or degradation of reagents. Quality control charts should be maintained to follow the performance of the assay.

### 6.0 SPECIMEN COLLECTION AND PREPARATION

Measurement of serum thyroxine concentration is generally recommended for the diagnosis of thyroid disease and monitoring of treatment. Thyroxine concentration permits construction of a graph of activity against thyroxine concentration of an unknown serum sample. Significant improvement in assay methodology that has occurred allows for the employment of several serum reference calibrator with optimum sensitivity while requiring few technical manipulations. This in method, serum reference calibrator, patient specimen, or control is first added to a microplate well. Enzyme-T4 conjugate is added, and then the reagents are mixed. A reaction competition occurs between these two immunochemical reaction systems.

The employment of all serum reference calibrator with known thyroxine concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, thyroxine concentration permits construction of a graph of activity versus thyroxine concentration.

### 6.0 CALCULATION OF RESULTS

1. Results should be read within thirty minutes after substrate addition for maximum performance of the assay. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION.
2. Incubate at room temperature for fifteen (15) minutes.
3. Add 0.050 ml (50 µl) of stop solution to all wells and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
4. Read absorbance within thirty minutes after addition of stop solution.

Note: For reasing specimens with concentrations greater than 25 µg/dl, pipet 12.5µl of the specimen and 12.5µl of the 0.0 reference serum calibrator into the sample well (this maintains a uniform protein concentration). Multiply the test value by 2 to obtain the thyroxine concentration.

### 9.0 TEST PROCEDURE

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 microplate’s wells for each serum reference calibrator, control, and patient specimen to be assayed in duplicate. Replace any unused microplate strips in the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.020 ml (20 µl) of the appropriate serum reference calibrator or control into the assigned well.
3. Add 0.100 ml (100 µl) of Working Reagent A (T4 Enzyme Conjugate Solution) to all wells (see Reagent Preparation Section).
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 30 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.305ml (305 µl) of wash buffer (see Reagent Preparation Section) to each well and gently mix for 15-20 seconds. Add 0.100 ml (100 µl) of working substrate solution to all wells (see Reagent Preparation Section). Store diluted substrate solution at 2-8°C.
8. Add 0.100 ml (100 µl) of working substrate solution to all wells (see Reagent Preparation Section). Add 0.020 ml (20 µl) of stop solution to all wells and gently mix for 15-20 seconds.
9. Read absorbance within thirty minutes after addition of stop solution.

Note: *The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a standard curve prepared with each assay.*
11.0 QC PARAMETERS

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

1. The absorbance (OD) of calibrator 0 µg/dl should be > 1.3.

2. Four out of six quality control pools should be within the expected ranges (±2 σ).

3. Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. Thyroxine binding globulin concentration, and the binding of thyroxine to TBG. Thus, total thyroxine concentration alone is not sufficient to assess clinical status.

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9. Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. Thyroxine binding globulin concentration, and the binding of thyroxine to TBG. Thus, total thyroxine concentration alone is not sufficient to assess clinical status.

10. It is important to keep in mind that establishment of a range of values that can be expected to be found by a given method for a population of “normal” persons is dependent upon a multiplicity of factors: the specialty of the medical specialist, the population studied, the technique used, etc. Therefore, any laboratory should be able to establish the range of expected values based on their own experience and data collected from their own patients.

11.0.1 Expected Ranges of Values

A study of euthyroid adult population was undertaken to determine expected ranges for the Rapid T4 AccuBind® ELISA Test System. The mean (X) values, standard deviations (σ) and expected ranges (±2 σ) are presented in Table 1.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross-Reaction</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroxine</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>D-Thyroxine</td>
<td>0.9800</td>
<td>10 µg/dl</td>
</tr>
<tr>
<td>D3-Thyroxine</td>
<td>0.0150</td>
<td>100 µg/dl</td>
</tr>
<tr>
<td>D4-Thyroxine</td>
<td>0.0300</td>
<td>100 µg/dl</td>
</tr>
<tr>
<td>D5-Thyroxine</td>
<td>0.0001</td>
<td>100 µg/dl</td>
</tr>
<tr>
<td>D6-Thyroxine</td>
<td>0.0001</td>
<td>100 µg/dl</td>
</tr>
<tr>
<td>D7-Thyroxine</td>
<td>0.0001</td>
<td>100 µg/dl</td>
</tr>
</tbody>
</table>

15.0 REFERENCES


11. Table: T4 Values in µg/dl

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low</th>
<th>Normal</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>5.76</td>
<td>9.41</td>
<td>16.18</td>
</tr>
</tbody>
</table>

As measured in ten experiments in duplicate over a ten day period.

12.4 Sensitivity

The Rapid T4 AccuBind® ELISA Test System has a sensitivity of 3.2ng/ml. This is equivalent to a sample containing a concentration of 0.128 µg/dl. The sensitivity was ascertained by determining the variability of the 0 µg/dl serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

12.5 Precision

The within and between assay precision of the Rapid T4 AccuBind® ELISA Test System was determined on three different levels of pool control sera. The number (N), mean values (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

Table 2: Within Assay Precision (Values in µg/dl)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low</th>
<th>Normal</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>8.07</td>
<td>9.05</td>
<td>13.13</td>
</tr>
</tbody>
</table>

12.6 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. Other factors, such as patient history and all other clinical findings, must be considered.

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

4. 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.

5. 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.

6. Accurate and precise pipetting, as well as following the good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

7. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - is performed by a skilled individual or trained professional.

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

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

10. The absorbance (OD) of calibrator 0 µg/dl should be > 1.3.

11. Four out of six quality control pools should be within the expected ranges (±2 σ).

12. It is important to keep in mind that establishment of a range of values that can be expected to be found by a given method for a population of “normal” persons is dependent upon a multiplicity of factors: the specialty of the medical specialist, the population studied, the technique used, etc. Therefore, any laboratory should be able to establish the range of expected values based on their own experience and data collected from their own patients.