**1.0 INTRODUCTION**

**Intended Use:** The Quantitative Determination of Total Triiodothyronine Concentration in T3 Calibrators or Plasma by a Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of serum triiodothyronine concentration is generally regarded as a valuable tool in the diagnosis of thyroid dysfunction. This importance has provided the impetus for the discovery of blocking agents to the T3 binding serum in the last two decades. The advent of monospecific antiserum has provided the impetus for the development of procedure which enable triiodothyronine for a limited number of antibody concentrations, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. T3 Calibrators References – 1ml/vial - Icons A-F
B. T3 Enzyme Reagent – 1.5ml/vial - Icon E
C. T3/4 Conjugate Buffer – 13ml - Icon E
D. T3 Antibody Coated Plate – 96 wells - Icon F
E. Wash Buffer Concentrate – 20ml - Icon F
F. Substrate A – 7ml/vial - Icon S
G. Substrate B – 7ml/vial - Icon S
H. Stop Solution – 8ml/vial - Icon S

**Note 2:** Do not use reagents that are contaminated or have expired. For each assay, reagents should be used within thirty (30) minutes of adding the stop solution.

9.0 TEST PROCEDURE

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

9.0 TEST PROCEDURE

2.0 Introduce the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).

**Note:** Reagents should be performed by a skilled individual or trained professional.

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate.
2. Replace any unused microwell strips back into the BioSafety/medical laboratory.
3. Add 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
4. Add 0.100 ml (100µl) of Working Reagent A, T3 Enzyme Reagent to all wells (see Reagent Preparation Section).
5. Swirl the microplate gently for 20-30 seconds to mix and cover.
6. Incubate 60 minutes at room temperature.
7. Discard the contents of the microplate by decantation or aspiration. If decanting, blow the contents dry with absorbent paper.
8. Add 350µl 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 semi-automatic manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
9. Inoculate from room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between tests.

11. 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.

**Note:** For re-assaying specimens with concentrations greater than 7.5ng/ml, pipette 25µl of the specimen and 25µl of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout by 2 to obtain the triiodothyronine concentration.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of triiodothyronine 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 corresponding T3 concentration in ng/ml for linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of T3 for an unknown, locate the average absorbance of the duplicates for each unknown on the y-axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal (x-axis) of the graph (the duplicates of the unknown may be averaged as indicated in the following example, the average absorbance (1.130) intersects the dose response curve at 1.95ng/ml T3 concentration (See Figure 1).

**Note:** Computer data reduction software designed for ELISA assays may be used for the data reduction. If such
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve. In order for the assay results to be considered valid the response curve prepared with each assay should not illustrate only and accuracy should not be ascertained.

1. It is important that the time of reaction in each well is held between 1.50 and 2.00 minutes to avoid assay drift.

1.50

2.00

1.638

1.701

1.646

2.128

1.846

1.678

1.864

1.662

Cal A

Cal B

Cal C

Cal D

Cal E

Cal F

Ctrl 1

Ctrl 2

Patient

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 0 ng/ml should be ≥ 1.3.

2. Four out of six quality control plates 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

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

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

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

4. If more than one 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 sol. 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 aspiration or decantation wash 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 specimens with T3 concentrations above 7.5 ng/mL may be diluted ½ with ‘0’ serum reference. The sample’s concentration is obtained by multiplying the result by the dilution factor.

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 and/or the automated instruments used with this device, and to perform routine preventative maintenance.

13. Risk Analysis – as required by CE Mark IVDD 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 alone are only one aspect for determining patient care and should not be the sole basis for therapy.

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 produce false test results, or if results are incorrectly interpreted, 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 reference.

6. Total serum triiodothyronine concentration is dependent upon a multiplicity of factors: the specificity of the method, the population tested 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 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 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 precisions of the T3 AccuBind™ ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (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>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>10</td>
<td>0.76</td>
<td>0.07</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>1.85</td>
<td>0.13</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>3.43</td>
<td>0.16</td>
</tr>
</tbody>
</table>

14.2 Sensitivity

The T3 AccuBind™ ELISA test system has a sensitivity of 0.04 ng/mL. The sensitivity was assessed by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The T3 AccuBind™ ELISA method was compared with a reference radioimmunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The mean range was 7.5 – 8.0 ng/ml). The total number of such specimens was 120. The least square regression equation (y = mx+b) and the correlation coefficient were computed for the T3 AccuBind™ ELISA method in comparison with the reference method. The data obtained is presented in Table 4.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean</th>
<th>Least Square Regression</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3 AccuBind™ ELISA</td>
<td>1.62</td>
<td>y = 3.8 + 0.947(x)</td>
<td>0.987</td>
</tr>
</tbody>
</table>

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

14.4 Specificity

The cross-reactivity of the triiodothyronine antibody to selected substances was evaluated by adding one interfering substance to a serum matrix at various concentrations. The within and between assay precisions of the T3 AccuBind™ ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (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>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
<th>Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-Triiodothyronine</td>
<td>&lt; 0.0002</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>I-Thyroxine</td>
<td>&lt; 0.0001</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>Iodothyronine</td>
<td>&lt; 0.0001</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>Diiodothyronine</td>
<td>&lt; 0.0001</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>Phenylnitrate</td>
<td>&lt; 0.0001</td>
<td>10 µg/ml</td>
</tr>
</tbody>
</table>

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

9. Patient specimens with T3 concentrations above 7.5 ng/mL may be diluted ½ with ‘0’ serum reference. The sample’s concentration is obtained by multiplying the result by the dilution factor.

10.1 Reference


Sterling, L., "Diagnosis and Treatment of Thyroid Disease", Cleveland Press (1983).


