2. Control materials or serum pools should be included in each run to monitor the performance of the assay.

3. T3 Calibrators

- **Calibrator A**: 0 ng/mL (0.0 mL)
- **Calibrator B**: 0.5 ng/mL (0.5 mL)
- **Calibrator C**: 1 ng/mL (0.5 mL)
- **Calibrator D**: 3 ng/mL (0.5 mL)
- **Calibrator E**: 10 ng/mL (0.5 mL)

4. **Contents**: One bottle containing 5 T3 calibration standards, each in a reconstituted protein-based buffer.

5. **Storage**: Refrigerate at 2–8°C, or as indicated on the label. Once opened, standards should be used within 14 days or aliquoted and stored frozen.

6. **Volume**: 6 mL/bottle

7. **Contents**: One bottle containing 1M sulfuric acid.

8. **Storage**: Refrigerate at 2–8°C

9. **Volume**: 15 mL/bottle

10. **Contents**: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

11. **Storage**: Refrigerate at 2–8°C

12. **Volume**: 16 mL/bottle

13. **Contents**: One bottle containing a protein-based buffer with a non-ionic detergent and a non-mercury preservative.

14. **Storage**: Refrigerate at 2–8°C

15. **Volume**: 15 mL/bottle

16. **Contents**: One bottle containing T3 in a protein-based buffer with a non-mercury preservative.

17. **Storage**: Refrigerate at 2–8°C

18. **Volume**: 50 mL/bottle

19. **Stability**: 12 months or as indicated on label.

20. **Calibrator Concentration Volume**

- **Calibrator A**: 6 mg/mL 2.0 mL
- **Calibrator B**: 0.2 mg/mL 0.5 mL
- **Calibrator C**: 1 mg/mL 0.5 mL
- **Calibrator D**: 3 mg/mL 0.5 mL

21. **Storage**: Refrigerate at 2–8°C

22. **Stability**: 12 months or as indicated on label.

**POTENTIAL BIOHAZARDOUS MATERIAL**

- Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling human specimens.

**CHEMICAL HAZARDS**

- Avoid contact with reagents containing TMK, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMK is a suspected carcinogen.

**SPECIMEN COLLECTION AND STORAGE**

- Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C for longer. Always perform the assay within 48 hours from the date printed on the label. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

**SPECIMEN PRETREATMENT**

This assay is a direct system; no specimen pretreatment is necessary.

**REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED**

1. Pipette to dispense 50, 100, 150 and 300 μL

2. Pipette tips

3. Disposable pipette tips

4. Distilled or deionized water

5. Plate shaker

6. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

**REAGENTS PROVIDED**

1. **Rabbit Anti-T3 Antibody-Coated Break-Apart Well Microplate** — Ready To Use

2. **Contents**: One 96-well (1248) polyclonal antibody-coated microplate in a resealable pouch with desiccant.

3. **Storage**: Refrigerate at 2–8°C

4. **Stability**: 12 months or as indicated on label.

5. **T3-Horseradish Peroxidase (HRP) Conjugate**

- **Contents**: T3-HRP conjugate in a protein-based buffer with a non-mercury preservative.

6. **Storage**: Refrigerate at 2–8°C

7. **Stability**: 12 months or as indicated on label.

8. **Storage Preparations**

- **Preparation**: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

9. **Assay Buffer** — Ready To Use

- **Contents**: One bottle containing a protein-based buffer with a non-ionic detergent and a non-mercury preservative.

10. **Storage**: Refrigerate at 2–8°C

11. **Stability**: 12 months or as indicated on label.

**PROCEDURAL CAUTIONS AND WARNINGS**

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained if strict and careful adherence to the instructions provided.

2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.

3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.

4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.

5. All kit reagents and specimens should be brought to room temperature and mixed thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.

6. A calibrator curve must be established for every run.

7. The controls should be included in every run and fall within established confidence limits.

8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.

9. When reading the microplates, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

10. The assay buffer is sensitive to light and should be stored in original dark glass containers from direct sunlight.

11. When dispensing the substrate and stopping solution, do not dark dye dark and discard any that is left over.

12. When pipetting in these procedures, the pipette tip is to be used dilute 240 μL of HRP in 12 μL of HRP in 2 mL of assay buffer. If the whole plate is to be used dilute 50 mL of assay buffer concentrate in 450 mL of water.

13. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.

14. Do not mix vials of different concentrations of reagents together and do use an unspent component before the expiration date printed on the label.

15. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

**LIMITATIONS**

1. All the reagents within the kit are calibrated for the direct determination of T3 in human serum. The kit is not calibrated for the determination of T3 in other specimens of human origin.

2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.

3. Any samples or control sera containing acide or thimerosal are not compatible with this kit, as they may lead to false negative results.

4. Only calibrator A may be used to dilute all high serum samples. The use of any other reagent may lead to false results.

5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. In the absence of concurrent assay results, the final decision on the diagnosis and treatment of thyroid related diseases, including thyroid cancer, should be determined by a practitioner who is familiar with all relevant background information.

6. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. In the absence of concurrent assay results, the final decision on the diagnosis and treatment of thyroid related diseases, including thyroid cancer, should be determined by a practitioner who is familiar with all relevant background information.

7. SAFETY CAUTIONS AND WARNINGS

**POTENTIAL BIOHAZARDOUS MATERIAL**

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. Therefore kit users should consider a potential low risk of infection and handle with the same precautions as applied to any biological reagent.

**CHEMICAL HAZARDS**

Avoid contact with reagents containing TMK, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMK is a suspected carcinogen.

**SPECIMEN COLLECTION AND STORAGE**

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C for longer. Always perform the assay within 48 hours from the date printed on the label. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

**SPECIMEN PRETREATMENT**

This assay is a direct system; no specimen pretreatment is necessary.

**REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED**

1. Pipette to dispense 50, 100, 150 and 300 μL

2. Pipette tips

3. Disposable pipette tips

4. Distilled or deionized water

5. Plate shaker

6. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

**REAGENTS PROVIDED**

1. **Rabbit Anti-T3 Antibody-Coated Break-Apart Well Microplate** — Ready To Use

2. **Contents**: One 96-well (1248) polyclonal antibody-coated microplate in a resealable pouch with desiccant.

3. **Storage**: Refrigerate at 2–8°C

4. **Stability**: 12 months or as indicated on label.

5. **Wash Buffer Concentrate** — Requires Preparation

- **Contents**: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

- **Storage**: Refrigerate at 2–8°C

- **Volume**: 50 mL/bottle

- **Stability**: 12 months or as indicated on label.

6. **Assay Buffer** — Ready To Use

- **Contents**: One bottle containing a protein-based buffer with a non-ionic detergent and a non-mercury preservative.

- **Storage**: Refrigerate at 2–8°C

- **Volume**: 15 mL/bottle

- **Stability**: 12 months or as indicated on label.

7. **TMB Substrate** — Ready To Use

- **Contents**: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

- **Storage**: Refrigerate at 2–8°C

- **Volume**: 16 mL/bottle

- **Stability**: 12 months or as indicated on label.

8. **Stopping Solution** — Ready To Use

- **Contents**: One bottle containing 1M sulfuric acid.

- **Storage**: Refrigerate at 2–8°C

- **Volume**: 6 mL/half bottle

- **Stability**: 12 months or as indicated on label.
5. If a sample reads more than 10 ng/mL, then dilute it with
3. Calculate the mean optical density of each unknown
calibrator curve. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.64</td>
<td>0.07</td>
<td>10.4</td>
</tr>
<tr>
<td>2</td>
<td>1.24</td>
<td>0.12</td>
<td>9.7</td>
</tr>
<tr>
<td>3</td>
<td>4.86</td>
<td>0.44</td>
<td>9.0</td>
</tr>
</tbody>
</table>

RECOVERY
Spiked samples were prepared by adding defined amounts of T3 to a serum pool. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Unspiked</td>
<td>1.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ 2.0</td>
<td>3.0</td>
<td>3.3</td>
<td>115.1</td>
</tr>
<tr>
<td>+ 3.3</td>
<td>5.0</td>
<td>4.6</td>
<td>108.7</td>
</tr>
<tr>
<td>+ 5.0</td>
<td>5.7</td>
<td>6.3</td>
<td>90.5</td>
</tr>
</tbody>
</table>

LINEARITY
Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.90</td>
<td>1.65</td>
<td>103.4</td>
</tr>
<tr>
<td>1/2</td>
<td>1.50</td>
<td>0.73</td>
<td>97.3</td>
</tr>
<tr>
<td>1/4</td>
<td>0.71</td>
<td>0.36</td>
<td>51.1</td>
</tr>
<tr>
<td>1/8</td>
<td>0.40</td>
<td>0.24</td>
<td>60.0</td>
</tr>
<tr>
<td>2</td>
<td>5.10</td>
<td>2.55</td>
<td>102.0</td>
</tr>
<tr>
<td>1/2</td>
<td>2.60</td>
<td>1.28</td>
<td>93.8</td>
</tr>
<tr>
<td>1/4</td>
<td>1.20</td>
<td>0.64</td>
<td>125.0</td>
</tr>
<tr>
<td>1/8</td>
<td>0.60</td>
<td>0.36</td>
<td>111.1</td>
</tr>
<tr>
<td>3</td>
<td>8.00</td>
<td>4.00</td>
<td>112.3</td>
</tr>
<tr>
<td>1/2</td>
<td>4.45</td>
<td>2.00</td>
<td>115.0</td>
</tr>
<tr>
<td>1/4</td>
<td>2.30</td>
<td>1.00</td>
<td>100.0</td>
</tr>
</tbody>
</table>

EXPECTED VALUES
As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

<table>
<thead>
<tr>
<th>Group</th>
<th>Range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Normal Males and Females</td>
<td>0.7–2.1</td>
</tr>
</tbody>
</table>

INTER-ASSAY PRECISION
Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.64</td>
<td>0.07</td>
<td>10.4</td>
</tr>
<tr>
<td>2</td>
<td>1.24</td>
<td>0.12</td>
<td>9.7</td>
</tr>
<tr>
<td>3</td>
<td>4.86</td>
<td>0.44</td>
<td>9.0</td>
</tr>
</tbody>
</table>

CALCULATIONS
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or S-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 10 ng/mL, then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

FACTORY TESTS
1. Specimen Pretreatment: None.
2. All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.
3. Pipette 50 μL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 μL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. Wash the wells 3 times with 300 μL of diluted wash buffer and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 150 μL of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 10–15 minutes at room temperature.
9. Pipette 50 μL of stopping solution into each well at timed intervals.
10. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

PERFORMANCE CHARACTERISTICS
SENSITIVITY
The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the DBC Direct T3 ELISA kit is 0.16 ng/mL.

SPECIFICITY (CROSS-REACTIVITY)
The following compounds were tested for cross-reactivity with the Direct T3 ELISA kit with T3 cross-reacting at 100%.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Thyroxine</td>
<td>100</td>
</tr>
<tr>
<td>D-Thyroxine</td>
<td>34</td>
</tr>
<tr>
<td>Triiodothyropropionic acid</td>
<td>20</td>
</tr>
<tr>
<td>Dioiodo-D-thyroxine</td>
<td>0.5</td>
</tr>
<tr>
<td>D-Thyroxine</td>
<td>0.2</td>
</tr>
<tr>
<td>L-Thyroxine</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The following compounds were tested but cross-reacted at less than 0.1%: Dioiodothyrosine, lodothyrosine, Phenytoin, Sodium Salticyle and T3-thyrohormone.

INTRA-ASSAY PRECISION
Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.65</td>
<td>0.08</td>
<td>12.3</td>
</tr>
<tr>
<td>2</td>
<td>1.19</td>
<td>0.14</td>
<td>11.7</td>
</tr>
<tr>
<td>3</td>
<td>5.16</td>
<td>0.21</td>
<td>4.1</td>
</tr>
</tbody>
</table>

REFERENCES

Other related ELISA kits also available from DBC:
- DBC Direct Free T4 ELISA Kit, REF CAN-T4-4340
- DBC Direct Total T4 ELISA Kit, REF CAN-T4-4240
- DBC Direct TSH ELISA Kit, REF CAN-T4-4320
- DBC Direct TSH ELISA Kit, REF CAN-TSH-4080

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