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

2. Control materials or reagent 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 gently but 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 indicate errors which are not always as obvious as those that do not reflect established ranges.

9. When using a microtiter plate reader, the presence of bubbles in the well plate can affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

10. The substrate solution (TMB) is sensitive to light and peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.

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

13. Do not mix various lots of kit numbers within a test and do not use a component beyond the expiration date printed on the label.

14. Kit reagents must be regarded as hazardous waste and handled with the same precautions as applied to any animal origin.

CLINICAL APPLICATIONS

Thyroxine (T4), the primary thyroid hormone, circulates in blood almost completely bound to thyroxine-binding globulin (TBG) and human serum albumin (HSA). The binding sites on the thyroxine-binding globulin are of low binding capacity in order not to disturb the equilibrium between T4 and its carrying proteins. The assay is carried out under normal physiological conditions of pH, temperature and ionic strength.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of T4 in human serum. The kit is not calibrated for the determination of T4 in other specimens of human or animal origin.

2. Do not use grossly hemolyzed, grossly lipemic, icteric or protein-contaminated specimens.

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

4. Samples reading higher than 100 pg/mL should be reported as such and should not be diluted. Dilution will alter the estimated serum albumin concentrations and lead to false results.

5. The interpretation of T4 results can be complicated by a variety of drugs whose nonthyroidal illness and some rare conditions such as familial dysalbuminemic hyperthyroxinemia (FDH). For diagnostic purposes, the results of this assay should only be used in conjunction with the clinical examination, medical history and other diagnostic tests.

6. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patient who has received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

SAFETY CAUTIONS AND WARNINGS

Potential Biohazardous Material

Human serum that may be used in the preparation of antibodies for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HIV and Human Immunodeficiency Virus (HIV) and HCV and Hepatitis B virus. The main carriers of thyroxine are blood almost completely bound to carrier proteins. However, the labelled T4 (conjugate) employed in this assay system has shown no binding properties towards thyroxine-binding globulin and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.

When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.

PRECAUTIONS AND RECOMMENDATIONS

1. Mouse Anti-T4 Antibody-Coated Break-Apart Well Microplate — Ready To Use

Contents: One 96-well (12x8) monoclonal antibody-coated microplate in a ready-to-use disposable wash pouch with desiccant.

Storage: Refrigerate at 2–8°C

2. T4-Horseradish Peroxidase (HRP) Conjugate — Requires Preparation

Contents: T4-HRP conjugate in a ready-to-use matrix with a non-mercury preservative.

Volume: 50 μL/vial

Storage: Refrigerate at 2–8°C

3. Calibration Standards — Requires Preparation

Contents: Calibrator A 0 pg/mL, 0.5 mL; Calibrator B 2 pg/mL, 0.5 mL; Calibrator C 6 pg/mL, 0.5 mL; Calibrator D 20 pg/mL, 0.5 mL; Calibrator E 100 pg/mL, 0.5 mL

Storage: Refrigerate at 2–8°C

4. Controls — Ready To Use

Contents: Two vials containing T4 in a human serum-based matrix with a non-mercury preservative. Prepared by spiking serum with defined quantities of T4. For diagnostic purposes, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate — Requires Preparation

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

Volume: 5 L/bottle

Storage: Refrigerate at 2–8°C

6. Assay Buffer — Ready To Use

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.

Volume: 50 mL/bottle

Storage: Refrigerate at 2–8°C

7. TMB Substrate — Ready To Use

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO based matrix with a non-mercury preservative.

Volume: 6 mL/bottle

Storage: Refrigerate at 2–8°C

8. Stopping Solution — Ready To Use

Contents: One bottle containing 1M sulfuric acid.

Volume: 6 mL/bottle

Storage: Refrigerate at 2–8°C

9. Calibrators — Ready To Use

Contents: Five vials containing T4 in a human serum-based matrix with a non-mercury preservative. Prepared by spiking serum with a defined quantity of T4.

* Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator Concentration Volume

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Concentration</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0 pg/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>B</td>
<td>2 pg/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>C</td>
<td>6 pg/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>D</td>
<td>20 pg/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>E</td>
<td>100 pg/mL</td>
<td>0.5 mL</td>
</tr>
</tbody>
</table>

Storage: Refrigerate at 2–8°C

Stability: 12 months in unopened vials or as indicated on label. On opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

INTENDED USE

For the direct quantitative determination of Free Thyroxine by an enzyme immunoassay in human serum.
TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean OD</th>
<th>Value (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.043</td>
<td>2.094</td>
<td>2.068</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>1.886</td>
<td>1.973</td>
<td>1.929</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>1.709</td>
<td>1.727</td>
<td>1.718</td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>1.032</td>
<td>1.049</td>
<td>1.041</td>
<td>20</td>
</tr>
<tr>
<td>E</td>
<td>0.266</td>
<td>0.283</td>
<td>0.274</td>
<td>80</td>
</tr>
<tr>
<td>Unknown</td>
<td>1.332</td>
<td>1.312</td>
<td>1.322</td>
<td>13.0</td>
</tr>
</tbody>
</table>

TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in pg/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>4.27</td>
<td>0.53</td>
<td>12.3</td>
</tr>
<tr>
<td>2</td>
<td>20.54</td>
<td>2.36</td>
<td>11.5</td>
</tr>
<tr>
<td>3</td>
<td>67.34</td>
<td>6.67</td>
<td>9.9</td>
</tr>
</tbody>
</table>

EXPECTED NORMAL VALUES

For all clinical assays each laboratory should collect data and establish their own range of expected normal values. The following reference range (pg/mL) was established with 80 apparently healthy adults:

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Range (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>80</td>
<td>7–22</td>
</tr>
</tbody>
</table>

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 fT4 ELISA kit is 1.6 pg/mL.

Specificity (Cross-Reactivity)

The following compounds were tested for cross-reactivity with the Direct fT4 ELISA kit with T4 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>94</td>
</tr>
<tr>
<td>3.3,5,5'-Triiodo-L-Thyronine</td>
<td>86</td>
</tr>
<tr>
<td>Reverse T3</td>
<td></td>
</tr>
<tr>
<td>3.3,5-Triodo-L-Thyronine (T3)</td>
<td>3.3</td>
</tr>
<tr>
<td>3.3,5-Trido-D-Thyroxine</td>
<td>1.8</td>
</tr>
<tr>
<td>3.3,5-Diiodothyronilic acid</td>
<td>0.8</td>
</tr>
<tr>
<td>3.3,5-Diiodo-L-Thyronine</td>
<td></td>
</tr>
<tr>
<td>3.5-Diiodo-L-Tyroxine</td>
<td></td>
</tr>
<tr>
<td>3-Iodo-L-Tyrosine</td>
<td></td>
</tr>
</tbody>
</table>

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in pg/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>3.79</td>
<td>0.16</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>23.26</td>
<td>1.14</td>
<td>4.9</td>
</tr>
<tr>
<td>3</td>
<td>70.60</td>
<td>3.04</td>
<td>4.3</td>
</tr>
</tbody>
</table>

INTERRUPTION

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.

REFERENCES


OTHER RELATED DBC ELISA KITS

- DBC Direct Total T4 ELISA Kit: CAN-T4-4240
- DBC Direct Total T3 ELISA Kit: CAN-T3-4220
- DBC Direct Free T4 ELISA Kit: CAN-T4-4230
- DBC Direct TSH ELISA Kit: CAN-TSH-4080

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