TOTAL TRIIODOTHYRONINE (T3) STREPTAVIDIN ELISA

Intended Use:
For the Quantitative Determination of Total Triiodothyronine Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay.

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 significant improvement in assay methodology that has occurred in the last two decades. The advent of monospecific antiserum and the discovery of blocking agents to the T3 binding serum proteins have enabled the development of procedurally simple radioimmunoassays (1,2).

Principle:
Competitive Enzyme Immunoassay
The essential reagents required for a solid phase enzyme immunoassay include antibody, conjugate and native antigen. Upon adding antibody, 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 binding sites. The interaction is illustrated by the following equation:

\[ E + Ag \rightarrow EAg \]

\[ EAg + A = EAgA \]

\[ EAgA + Ag = EAgAg \]

\[ EAgAg + Ag = EAgAgAg \]

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

The enzyme activity in the antibody bound fraction is measured by reaction with a suitable substrate to produce colour, which is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Reagents:
Materials Provided: Store at 2-8°C

<table>
<thead>
<tr>
<th>(T3) Kit Contents</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Serum of 6 levels as mentioned on the label</td>
<td>6x1.0ml</td>
</tr>
<tr>
<td>Total T3 Enzyme conjugate</td>
<td>1x1.5ml</td>
</tr>
<tr>
<td>T3/T4 Conjugate buffer</td>
<td>1x13ml</td>
</tr>
<tr>
<td>T3 Biotin Reagent</td>
<td>1x7ml</td>
</tr>
<tr>
<td>Streptavidin coated Microplate</td>
<td>96 Wells</td>
</tr>
<tr>
<td>Wash Solution Concentrate</td>
<td>1x20ml</td>
</tr>
<tr>
<td>Substrate A</td>
<td>1x7ml</td>
</tr>
<tr>
<td>Substrate B</td>
<td>1x7ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1x8ml</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1 piece</td>
</tr>
</tbody>
</table>

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C.

Note 3: Above reagents are for a single 96-well microplate

Materials Required But Not Provided:
1. Pipette capable of delivering 50μl volumes with a precision of better than 1.5%.
2. Dispensers (for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200μl) and (200-1000μl) dispensers (for conjugate and substrate dilutions.
4. Micropipette washes a squeeze bottle (optional).
5. Micropipette with 450ml and 600ml wavelength absorbance capability.
6. Test tubes for dilution of enzyme conjugate and substrate A and B.
7. Absorbent Paper for blotting the microplate wells.

8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. Quality control materials.

Precautions:
For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals
All product(s) that contain human serum or human tissue cannot be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies. 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.

Specimen Collection And Preparation:
The specimens shall be blood, serum and plasma. The blood should be allowed to clot. Centrifuge the serum to separate the serum from the cells.

Note 1: Specimen(s) may be refrigerated at 2-8°C for a maximum period of 48 hours. If the specimen(s) cannot be assayed within 48 hours, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Before assay, allow the specimens to equilibrate to ambient temperature (20°C - 27°C) when assayed in duplicate. 0.10ml of the specimen is required.

Reagent Preparation:
1. Working Reagent A - T3-enzyme Conjugate Solution
Dilute the T3-enzyme conjugate 1:11 with all Total T3/T4 conjugate buffer in a suitable container. For example, dilute 160μl of conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within a period of twenty-four hours for maximum performance of the assay.

2. Working Reagent B - T3 Biotin Reagent
Dilute co-reactive conjugate solution to all wells (see Reagent Preparation Section).

3. Working Reagent C - Working Substrate Solution
Add 0.050 ml (50μl) of the appropriate serum reference, control or specimen to be assayed in duplicate.

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

5. Add 0.050 ml (50μl) of T3 Biotin Reagent to all wells.

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

7. Incubate 60 minutes at room temperature.

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

9. Add 300μl 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 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.

10. Add 0.100 ml (100μl) of working signal reagent solution to all wells (see Reagent Preparation Section).

Always add reagents in the same order to minimize reaction time differences between wells.

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

11. Incubate at room temperature for fifteen (15) minutes.

12. Pipette 0.050ml (50μl) of the 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 wells.

13. Read absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. Results should be read within thirty (30) minutes of adding the stop solution.

Note: For re-asaying specimens with concentrations greater than 10.0ng/ml, pipette 25μl of the specimen and 25μl of the 0.0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the triiodothyronine total.

Quality Control: Each laboratory should assay external controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate uncritical change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

Results:

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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 on 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 vertical axis (y-axis) of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis (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).

The data presented in Example 1 and Figure 1 are for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

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 > 1.2.
2. Four out of six quality control pools should be within the established ranges.

Limitations Of Procedure:

A. Assay Performance:
1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time-deviation during reaction.
5. Plate readers measure vertical distances. Do not touch the bottom of the wells.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.

B. Interpretation:
1. 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 concentrations.
2. Total serum triiodothyronine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of triiodothyronine to TBG (3. 4). Thus, total triiodothyronine concentration alone is not sufficient to assess clinical status.
3. A decrease in total triiodothyronine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates.

Expected Ranges Of Values:

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Number</th>
<th>Abs (A)</th>
<th>Mean Abs (B)</th>
<th>Value (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL A</td>
<td>A1</td>
<td>3.046</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>3.046</td>
<td>3.046</td>
<td>0</td>
</tr>
<tr>
<td>CAL B</td>
<td>C1</td>
<td>2.374</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D1</td>
<td>2.378</td>
<td>2.376</td>
<td>0.5</td>
</tr>
<tr>
<td>CAL C</td>
<td>E1</td>
<td>2.046</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>2.048</td>
<td>2.047</td>
<td>1</td>
</tr>
<tr>
<td>CAL D</td>
<td>H1</td>
<td>1.596</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>1.599</td>
<td>1.598</td>
<td>2.5</td>
</tr>
<tr>
<td>CAL E</td>
<td>B2</td>
<td>1.225</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>1.225</td>
<td>1.225</td>
<td>5</td>
</tr>
<tr>
<td>CAL F</td>
<td>D2</td>
<td>0.886</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>0.888</td>
<td>0.888</td>
<td>10</td>
</tr>
</tbody>
</table>

**Patient**

| F2        | 1.226   | 1.225   |              |

*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.*

C. Specificity

The cross-reactivity of the triiodothyronine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of triiodothyronine needed to displace the same amount of tracer.

**Instruments And Application:**

Fortress Diagnostics' immunoassay products are designed to work in both manual and automated lab environments and are compatible with any open-end instrumentation, including chemistry analyzers, microplate readers and microplate washers. There may or may not be an application developed for your particular instrument please contact info@fortressdiagnostics.com

**Reference:**


**Table 2 and Table 3.**