INTENDED USE
ELECTRA™ T3 CLIA test is intended for the quantitative determination of Total Triiodothyronine (T3) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION
The hormones thyroxine (T4) and triiodothyronine (T3) circulate in the bloodstream, mostly bound to the plasma protein, thyroxine binding globulin (TBG). The concentration of T3 is much less than that of T4, but its metabolic potency is much greater. T3 determination is an important factor in the diagnosis of thyroid disease. Its measurement has uncovered a variant of hyperthyroidism in thyrotropic patients with elevated T3 values and normal T4 values. T3 determination is also useful in monitoring both patients under treatment for hyperthyroidism and patients who have discontinued anti-thyroid drug therapy. In addition to hyperthyroidism, T3 levels are elevated in women who are pregnant and in women receiving oral contraceptives or estrogen treatment.

PRINCIPLE
ELECTRA™ T3: Quantitative CLIA assay is for use on ELECTRA analyzers. ELECTRA™ T3 CLIA works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalysed by HRP, the oxidation of luminal by hydrogen peroxide produces an electronically excited form of 3-aminoanthiphalate which on relaxation emits light with maximum intensity at λ=425nm.

In ELECTRA™ T3 CLIA, a certain amount of anti-T3 antibody is coated on microtiter wells. A measured amount of patient serum, and a constant amount of T3 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, T3 in the samples and conjugated T3 compete for the limited binding sites on the anti-T3 antibody of the wells. After incubation, the wells are washed by wash Buffer to remove unbound T3 conjugate. A solution of chemiluminescent substrate is then added and Luminescence is measured in RLU. The intensity of the emitting light is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T3 in the sample. By reference to a series of T3 standards assayed in the same way, the concentration of T3 in the unknown sample is quantified.

MATERIALS & COMPONENTS
Materials provided with the test kits:
- Coated Micro Wells: Micro wells coated with Anti-T3 antibody.
- T3 HRPO Conjugate Diluent.
- T3 HRPO Enzyme Conjugate (20X)
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxidase solution.
- T3 Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X)

Materials required but not provided:
- Precision pipettes: 10-100μl, 50-200μl, 100-1000μl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELECTRA™ Analyzer

STORAGE AND STABILITY
1. ELECTRA™ T3 kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated micro-wells should be used within one month of opening the pouch. Once opened, the pouch must be sealed properly to protect from moisture. In case the desiccant pouch changes color from blue to white, the strips should not be used.
3. Diluted wash buffer is stable up to one week at 2-8°C.
4. Working Substrate (A+B) must be used immediately.

SPECIMEN COLLECTION
1. Collect blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination

PRECAUTIONS
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION
- All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
- Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- Dilute Enzyme Conjugate with Conjugate Diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.

<table>
<thead>
<tr>
<th>No. of Strips</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<th>10</th>
<th>11</th>
<th>12</th>
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<tbody>
<tr>
<td>T3 HRPO Enzyme Conjugate (20X) (µl)</td>
<td>12.5</td>
<td>25</td>
<td>50</td>
<td>75</td>
<td>100</td>
<td>125</td>
<td>150</td>
<td>175</td>
<td>200</td>
<td>225</td>
<td>250</td>
<td>275</td>
<td>300</td>
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<tr>
<td>T3 HRPO Conjugate Diluent (µl)</td>
<td>250</td>
<td>500</td>
<td>1000</td>
<td>1500</td>
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<td>2500</td>
<td>3000</td>
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3. Prepare a Working Substrate by Mixing Substrate A and Substrate B in equal volume (1:1 ratio) before addition to the micro-wells

<table>
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<tr>
<th>No. of Strips</th>
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<tbody>
<tr>
<td>Substrate-A µl</td>
<td>250</td>
<td>450</td>
<td>650</td>
<td>850</td>
<td>1050</td>
<td>1250</td>
<td>1450</td>
<td>1650</td>
<td>1850</td>
<td>2050</td>
<td>2250</td>
<td>2450</td>
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<tr>
<td>Substrate-B µl</td>
<td>250</td>
<td>450</td>
<td>650</td>
<td>850</td>
<td>1050</td>
<td>1250</td>
<td>1450</td>
<td>1650</td>
<td>1850</td>
<td>2050</td>
<td>2250</td>
<td>2450</td>
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TEST PROCEDURE
1. Secure the desired number of coated wells in the holder. Dispense 25µl of Standards & Serums into the appropriate wells.
2. Dispense 50µl of diluted Conjugate into each well. Incubate at room temperature (18-25°C) for 30 minutes.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto the absorbent paper or paper towels to remove all residual water droplets.
4. Add 50 µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
5. Cover the ELECTRA™ microplate and incubate for 10 minutes at room temperature (18-25°C) in dark.
6. Read the ELECTRA™ micro-plate exactly at 10 minutes in ELECTRA™ Analyzer. If ELECTRA™ micro-plate is not read between 10-15 minutes the test results should be considered as invalid.

CALCULATIONS
Construct a Standard curve by plotting the mean RLU obtained from each reference Standards against its concentrations in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of T3 in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve
Results of a typical standard run with RLU’s shown in the Y axis against T3 concentrations shown in the X axis. Suggest: Use 4-Parameter Standard curve to calculate sample values.

T3 Values (ng/ml) | RLU’s
---|---
A | 2003024
B | 1215377
C | 970818
D | 459054
E | 207446
F | 54856

This Standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain their own Standard curve and data.

Expected Ranges of values
Normal Range: 0.6-2.0 ng/ml
The minimal detectable concentration of T3 by this assay is estimated to be 0.25 ng/ml.

PERFORMANCE CHARACTERISTICS
A) Internal Evaluation:
1. Accuracy: In an internal study ELECTRA™ T3 was evaluated against commercially available licensed kit with 90 random clinical samples, & ELECTRA™ T3 has demonstrated 100% clinical correlation with the