T3 RIA (CT)

Radioimmunoassay for the quantitative measurement of human 3,5,3’ Triiodothyronine (T3) in serum.

REF MG13081

Σ 96

2-8 °C

EU: IVD

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I. **INTENDED USE**
Radioimmunoassay for the quantitative measurement of human 3,5,3’ Triiodothyronine (T3) in serum.

II. **GENERAL INFORMATION**
A. Proprietary name : T3 RIA (CT)
B. Catalog number : MG13081 : 96 tests

III. **CLINICAL BACKGROUND**
A. Biological activity
The thyroid gland exerts powerful and essential regulatory influences on growth, differentiation, cellular metabolism, and general hormonal balance, as well as on the maintenance of metabolic activity and the development of the skeletal and organ system. The hormones thyroxine (T4) and 3,5,3’ triiodothyronine (T3) circulate in the blood stream, 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.

B. Clinical applications
T3 determination is an important factor in the diagnosis of thyroid disease. Its measurement has uncovered a variant of hyperthyroidism in thyrotoxic patient with elevated T3 levels and normal T4 levels. An increase in T3 without an increase in T4 is frequently a forerunner of recurrent thyrotoxicosis in previously treated patients. In other patients, euthyroidism is attributable to normal T3, although their T4 values are subnormal.

T3 determination is also useful in monitoring both patient under treatment for hyperthyroidism and patients who have discontinued anti-thyroid drug therapy. It is especially valuable in distinguishing between euthyroid subjects.

In women, T3 levels are elevated during pregnancy, during estrogen treatment, and contraceptive hormone therapy. When T3 levels parallel TBG increases in a manner analogous to T4 levels, these changes are not a reflection of altered thyroid status.
A fixed amount of $^{125}\text{T}3$ labelled T3 competes with the T3 to be measured present in the sample or in the calibrator for a fixed amount of anti-T3 antibody sites, which are bound to the goat anti mouse antibodies immobilized to the wall of a polystyrene tube. After 1 hour incubation at room temperature, an aspiration step terminates the competition reaction. The tubes are then washed with 2 ml of working wash solution and aspirated again. A calibration curve is plotted and the T3 concentrations of the samples are determined by dose interpolation from the calibration curve.

### V. REAGENTS PROVIDED

<table>
<thead>
<tr>
<th>Reagents</th>
<th>96 Tests Kit</th>
<th>Colour Code</th>
<th>Recollection</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUBES</td>
<td>2 x 48</td>
<td>black</td>
<td>Ready for use</td>
</tr>
<tr>
<td>TRACER: $^{125}$Iodine labelled T3 (HPLC grade) in phosphate buffer with bovine casein and azide (&lt;0.1%)</td>
<td>1 vial 21 ml 111 kllq</td>
<td>red</td>
<td>Ready for use</td>
</tr>
<tr>
<td>CAL 0 LYO</td>
<td>1 vial lyophil. yellow</td>
<td>Add 0.5 ml distilled water</td>
<td></td>
</tr>
<tr>
<td>CAL N LYO</td>
<td>5 vials lyophil. yellow</td>
<td>Add 0.5 ml distilled water</td>
<td></td>
</tr>
<tr>
<td>Anti-T3 (monoclonal) antibodies in phosphate buffer with bovine serum albumin and thymol</td>
<td>1 vial lyophil. blue</td>
<td>Add 11 ml distilled water</td>
<td></td>
</tr>
<tr>
<td>WASHBUF CONC</td>
<td>1 vial 10 ml brown</td>
<td>Dilute 70 x with distilled water (use a magnetic stirrer).</td>
<td></td>
</tr>
<tr>
<td>CONTROL N LYO</td>
<td>2 vials lyophil. silver</td>
<td>Add 0.5 ml distilled water</td>
<td></td>
</tr>
</tbody>
</table>

Note: Use the zero calibrator for sera dilutions.

### VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Distilled water
2. Pipettes for delivery of: 50 µl, 100 µl, 200 µl, 500 µl and 2 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Tube shaker (700 rpm)
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional)
8. Any gamma counter capable of measuring $^{125}\text{T}$ may be used (minimal yield 70%).

### VII. REAGENT PREPARATION

**A. Calibrators:** Reconstitute the zero calibrator with 0.5 ml distilled water and the other calibrators with 0.5 ml distilled water.

**B. Controls:** Reconstitute the controls with 0.5 ml distilled water.

**C. Anti-T3:** Reconstitute the anti-T3 with 11 ml distilled water.

**D. Working Wash solution:** Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

### VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators are stable for 7 days at 2-8°C and controls are stable for 4 days at 2-8°C.
- Longer storage periods, aliquots should be made and kept at -20°C for maximum 3 months. Avoid subsequent freeze-thaw cycles.
- After reconstitution, the anti-T3 antibodies are stable for 6 weeks at 2-8°C.

**DO NOT FREEZE.**

- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

### IX. SPECIMEN COLLECTION AND PREPARATION

- Serum samples must be kept at 2-8°C.
- If the test is not run within 24 hrs., storage at -20°C is recommended.
- Avoid subsequent freeze-thaw cycles.

### X. PROCEDURE

**A. Handling notes**

Do not use the kit or components beyond expiry date.

Do not mix materials from different kit lots.

Bring all the reagents to room temperature prior to use.

Thoroughly mix all reagents and samples by gentle agitation or swirling.

Use a clean disposable pipette tip for addition of each different reagent and sample in order to avoid cross-contamination. High precision pipettes or automated pipetting equipment will improve the precision.

Respect the incubation times.

Prepare a calibration curve for each run, do not use data from previous runs.

**B. Procedure**

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes.

2. Briefly vortex calibrators, controls and samples and dispense 50µl of each into the respective tubes.

3. Dispense 200 µl of $^{125}$Iodine labelled T3 into each tube, including the uncoated tubes for total counts.

4. Dispense 100 µl of anti-T3 into each tube, except tubes for total counts.

5. Shake the tube rack gently by hand to liberate any trapped air bubbles.

6. Incubate for 1 hour at room temperature with continuous shaking.

7. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.

8. Wash tubes with 2 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.

9. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.

10. Count tubes in a gamma counter for 60 seconds.

### XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.

2. Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula:

   \[
   \text{B/B}_0(\%) = \left( \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \right) \times 100
   \]

3. Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B0(%) values for each calibrator point as a function of the T3 concentration of each calibrator point. Reject obvious outliers.

4. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

5. By interpolation of the sample (B/B0 (%)) values, determine the T3 concentrations of the samples from the calibration curve.

6. For each assay, the percentage of total tracer bound in the absence of unlabelled T3 (B0/T) must be checked.
**XII. TYPICAL DATA**

The following data are for illustration only and should never be used instead of the real time calibration curve.

<table>
<thead>
<tr>
<th>T3</th>
<th>cpm</th>
<th>B/Bo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total count</td>
<td>40019</td>
<td></td>
</tr>
</tbody>
</table>

Calibrator

<table>
<thead>
<tr>
<th>T3 (nmol/l)</th>
<th>cpm</th>
<th>B/Bo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>28572</td>
<td>100.0</td>
</tr>
<tr>
<td>0.35</td>
<td>24781</td>
<td>86.7</td>
</tr>
<tr>
<td>1.00</td>
<td>18112</td>
<td>63.4</td>
</tr>
<tr>
<td>2.50</td>
<td>10587</td>
<td>37.1</td>
</tr>
<tr>
<td>6.50</td>
<td>4629</td>
<td>16.2</td>
</tr>
<tr>
<td>14.00</td>
<td>2684</td>
<td>9.4</td>
</tr>
</tbody>
</table>

**XIII. PERFORMANCE AND LIMITATIONS**

**A. Detection limit**

The LoB was calculated by measuring the blank several times and calculating the 95th percentile of the distribution of the test values. The LoB was calculated to be 0.15 nmol/l.

The LoD was calculated as described in the guideline. The LoD was calculated to be 0.22 nmol/l.

The LoQ was calculated by testing 5 samples of low value 14 times in different test. The LoQ was calculated to be 0.22 nmol/l with CV of 20%.

**B. Specificity**

The percentage of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition is respectively:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-3,3',5'-triiodothyronine (L-T3)</td>
<td>100</td>
</tr>
<tr>
<td>3,3',5' - triiodothyronine (T3)</td>
<td>ND</td>
</tr>
<tr>
<td>L-thyroxine (L-T4)</td>
<td>0.17</td>
</tr>
<tr>
<td>D-thyroxine (D-T4)</td>
<td>0.04</td>
</tr>
<tr>
<td>3,3',5' - triiodothyroacetic acid (TRIAC)</td>
<td>52</td>
</tr>
<tr>
<td>3,5 - diiodo-L-tyrosine</td>
<td>0.22</td>
</tr>
</tbody>
</table>

ND = not detectable

Note: this table shows the cross-reactivity for the anti T3

**C. Precision**

**INTRA-ASSAY PRECISION**

<table>
<thead>
<tr>
<th>Serum</th>
<th>N</th>
<th>&lt;X&gt; ± SD (nmol/l)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>1.06 ± 0.05</td>
<td>4.7</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>5.49 ± 0.31</td>
<td>5.6</td>
</tr>
</tbody>
</table>

**INTER-ASSAY PRECISION**

<table>
<thead>
<tr>
<th>Serum</th>
<th>N</th>
<th>&lt;X&gt; ± SD (nmol/l)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>1.22 ± 0.05</td>
<td>3.7</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>5.43 ± 0.16</td>
<td>3.0</td>
</tr>
</tbody>
</table>

SD: Standard Deviation; CV: Coefficient of variation

**D. Accuracy**

**DILUTION TEST**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Theoretical Conc. (nmol/l)</th>
<th>Measured Conc. (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1/1</td>
<td>-</td>
<td>12.29</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>6.15</td>
<td>5.67</td>
</tr>
<tr>
<td></td>
<td>1/4</td>
<td>3.07</td>
<td>2.98</td>
</tr>
<tr>
<td></td>
<td>1/8</td>
<td>1.54</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>1/16</td>
<td>0.77</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Samples were diluted with zero calibrator.

**XIV. INTERNAL QUALITY CONTROL**

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Praties.

**XV. REFERENCE INTERVALS**

These values are given only for guidance; each laboratory should establish its own normal range of values.

T3 concentrations for untreated euthyroid subjects ranged from 1.7 to 2.9 nmol/l (n=80).

**XVI. PRECAUTIONS AND WARNINGS**

**Safety**

For *in vitro* diagnostic use only.

This kit contains 125I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in potentially infectious.

Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals.

None of the components contain animal substances which should be segregated to prevent cross contamination of different radioisotopes.

Bovine components originate from countries where BSE has not been reported.

All animal products and derivatives have been collected from healthy animals.

Local safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory.

Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HbsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections.

The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory.

Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HbsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections.

Therefore, handling of reagents, serum specimens should be in accordance with local safety procedures.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides.

During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.
XVII. BIBLIOGRAPHY


XVIII. SUMMARY OF THE PROTOCOL

<table>
<thead>
<tr>
<th>TOTAL COUNTS</th>
<th>CALIBRATORS</th>
<th>SAMPLE (S) CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>μl</td>
<td>μl</td>
<td>μl</td>
</tr>
<tr>
<td>Calibrators (0 to 5)</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Samples, Controls</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>Tracer</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Anti-T3</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

Incubation 1 hour at room temperature with continuous shaking

Separation Working Wash solution

Aspirate (or decant) 2.0 ml

Aspirate (or decant)

Counting Count tubes for 60 seconds

Revision nr : 170217/1
Revision date : 2017-02-17
Symbols of the kit components see MATERIALS SUPPLIED.

Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.

Voir MATERIEL FOURNI pour les symbôles des composants du kit.

Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.

Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.

Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.

Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER’S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.