I. INTENDED USE

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

II. GENERAL INFORMATION

A. Proprietary name: DIAsource T3-RIA-CT Kit

B. Catalog number:
- KIP1631: 96 tests
- KIP1634: 4 x 96 tests

C. Manufactured by: DIAsource ImmunoAssays S.A.
   Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

For technical assistance or ordering information contact:
Tel: +32 (0)10 84.99.11    Fax: +32 (0)10 84.99.91

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 125I labelled T3 competes with the T3 to be measured present in the sample or in the calibrator for a fixed amount of anti-T3 antibodies 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 Test Kit</th>
<th>4 x 96 Test Kit</th>
<th>Colour Code</th>
<th>Reconstitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubes coated with GAM (Goat anti Mouse)</td>
<td>2 x 48</td>
<td>8 x 48</td>
<td>black</td>
<td>Ready for use</td>
</tr>
<tr>
<td>Ag 125I</td>
<td>1 vial 21 ml 111 kBq</td>
<td>4 vials 21 ml 4x111 kBq</td>
<td>red</td>
<td>Ready for use</td>
</tr>
<tr>
<td>TRACER: 125I iodine labelled T3 (HPLC grade) in phosphate buffer with bovine casein and azide (&lt;0.1%)</td>
<td>1 vial lyophil.</td>
<td>2 vials lyophil.</td>
<td>yellow</td>
<td>Add 0.5 ml distilled water</td>
</tr>
<tr>
<td>Zero Calibrator in human serum and thymol</td>
<td>5 vials lyophil.</td>
<td>10 vials lyophil.</td>
<td>yellow</td>
<td>Add 0.5 ml distilled water</td>
</tr>
<tr>
<td>Anti-T3 (monoclonal) antibodies in phosphate buffer with bovine serum albumin and thymol</td>
<td>1 vial lyophil.</td>
<td>4 vials lyophil.</td>
<td>blue</td>
<td>Add 1 ml distilled water</td>
</tr>
<tr>
<td>Wash solution (TRS-HCl)</td>
<td>1 vial 10 ml</td>
<td>4 vials 10 ml</td>
<td>brown</td>
<td>Dilute 70 x with distilled water (use a magnetic stirrer)</td>
</tr>
<tr>
<td>Controls - N = 1 or 2 in human serum with thymol</td>
<td>2 vials lyophil.</td>
<td>4 vials lyophil.</td>
<td>silver</td>
<td>Add 0.5 ml distilled water</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 125I 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. For 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 50 µl of each into the respective tubes.
3. Dispense 200 µl of 125I 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 to aspirate (or decant) the content of each tube (except total counts).
8. Wash tubes with 2 ml Working Wash solution (except total counts) and aspirated again.
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:

   \[ \frac{B/B_0(\%)}{100} = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \]

3. Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the \( B/B_0(\%) \) 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 \( B/B_0(\%) \) 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 (B/0T) 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>
<tr>
<td>Calibrator</td>
<td>0.00 mmol/l</td>
<td>28572</td>
</tr>
<tr>
<td></td>
<td>0.35 mmol/l</td>
<td>24781</td>
</tr>
<tr>
<td></td>
<td>1.00 mmol/l</td>
<td>18112</td>
</tr>
<tr>
<td></td>
<td>2.50 mmol/l</td>
<td>10587</td>
</tr>
<tr>
<td></td>
<td>6.50 mmol/l</td>
<td>4629</td>
</tr>
<tr>
<td></td>
<td>14.00 mmol/l</td>
<td>2684</td>
</tr>
</tbody>
</table>

XIII. PERFORMANCE AND LIMITATIONS

A. Detection limit
Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was 0.1 nmol/l.

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 (rT3)</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

<table>
<thead>
<tr>
<th>INTRA-ASSAY PRECISION</th>
<th>INTER-ASSAY PRECISION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>N</td>
</tr>
<tr>
<td>A</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
</tr>
</tbody>
</table>

SD: Standard Deviation; CV: Coefficient of variation

D. Accuracy

<table>
<thead>
<tr>
<th>DILUTION TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Samples were diluted with zero calibrator.

<table>
<thead>
<tr>
<th>RECOVERY TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>12</td>
</tr>
</tbody>
</table>

To the best of our knowledge, no international reference material exists for this parameter.

E. Time delay between last calibrator and sample dispensing
As shown hereafter, assay results remain accurate even when a sample is dispensed 60 minutes after the calibrator has been added to coated tubes.

<table>
<thead>
<tr>
<th>TIME DELAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
</tr>
<tr>
<td>C 1</td>
</tr>
<tr>
<td>C 2</td>
</tr>
</tbody>
</table>

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 Practises.

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.

All radioactive handling should be executed in a designated area. Free from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radionuclides.

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.

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.

All animal products and derivatives have been collected from healthy animals. All Bovine components originate from countries where BSE has not been reported. 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. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

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

3. HOLLANDER, C.S., et al. (1972)
Clinical Laboratory Observation in Cases of T3 Toxicosis Confirmed by Radioimmunoassay.
Lancet., 1, 609-611.

T3 thyrotoxikosis; Thyrotoxikosis due to Elevated Serum Triiodothyronine levels.
Fertility and Sterility, 66, 6, 1033-1035.

Acta – Endocrinol.
77, 71.

XVIII. SUMMARY OF THE PROTOCOL

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

Incubation: 1 hour at room temperature with continuous shaking

Separation: Aspirate (or decant)
Tracer: 2.0 ml
Anti-T3: Aspirate (or decant)

Counting: Count tubes for 60 seconds

DiAsource Catalogue Nr: KIP1631
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