The T3 [I-125] RIA system provides a quantitative in vitro determination of L-3,5,3-thyriodothyronine (T3) in human serum in the range 0-12 nmol/L (0-780 ng/dL).

Introduction
Among the thyroid hormones produced in the thyroid gland triiodothyronin (T3) is regarded as the most biologically active molecule, produced by the deiodination of tetraiodothyronine (T4) in peripheral tissues. T3 is found in the bloodstream in a major (99.7 %) protein-bound, and a minor (0.3 %) unbound, fraction. Variations in total thyroid hormone in blood may result from either changes of binding proteins concentrations, or thyroid hormone production.

T3 contributes significantly to the maintenance of the euthyroid state, and the total T3 level has a role in screening for thyroid disease in conjunction with other tests. T3 alone cannot diagnose hyperthyroidism, but it may be more sensitive than T4 for hyperthyroidism.

Principle of method
This assay is based on the competition between unlabelled T3 and fixed quantity of I-125-labelled T3 for limited number of binding sites on T3 specific antibody. Allowing to react a fixed amount of tracer and antibody with different amounts of unlabelled ligand the amount of tracer bound by the antibody will be inversely proportional to the concentration of unlabelled ligand.

During a 1-hour incubation period with continuous agitation immuno-complex is immobilized on the reactive surface of test tubes. After incubation the reaction mixture is discarded, and the radioactivity is measured in a gamma counter. The concentration of antigen is inversely proportional to the radioactivity measured in test tubes. By plotting binding values against the standards can be found in the quality certificate sheet enclosed.

Sera and samples should be thawed and thoroughly mixed before assaying.

Preparation of reagents, storage
Store the reagents between 2-8 °C after opening. At this temperature each reagent is stable until expiry date of the kit. The actual expiry date is given on the package label and in the quality certificate. Open up both boxes of coated tubes at the same time.

CAUTION! Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure
(Far a quick guide, refer to Table 1.)
1. Prepare standard and controls, according to the dilution table above (Table 2).
2. Label coated tubes in duplicate for each standard (50-55), control sera (C1, CII) and samples (U). Optionally, label two test tubes for total counts (T).
3. Pipette 50 µl of each standard, control and samples into the properly labelled coated tubes.
4. Pipette 200 µl of tracer into each tube.
5. Fix the test tube rack firmly onto the shaker plate. Seal all tubes with a plastic foil. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or swirling in each tube (200 – 600 rpm).
6. Incube tubes for 1 hour at RT.
7. Add 1 ml distilled water to each tube.
8. Aspirate or decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
9. Calculate the T3 concentrations of the samples as described in calculation of results.

Calculation of results
The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2. The calculation of percent binding for each standard, control and sample respectively by using the following equation:

\[ \% \text{ Binding} = \left( \frac{B/B_0}{S_i} \right) \times 100 \]

Using semi-logarithmic graph paper plot B/B0 (%) for each standard versus the corresponding concentration of T3. A typical standard curve. Determine the T3 concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range. Out of fitting programs applied for computerized data processing logit-log, or spline fittings may be used.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microliters)

<table>
<thead>
<tr>
<th>T</th>
<th>S0-S5</th>
<th>C1/CII</th>
<th>Ux</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tracer</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Decant water</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Remove the water and blot on filter paper for 2 minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count radioactivity (60 sec/tube)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculate the results</td>
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<td></td>
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</tr>
</tbody>
</table>

*An incubation time of 30 minutes at room temperature is sufficient if the test is performed on automated RIA machine.
**Characterization of assay**

**Conversion of SI units** can be performed according to the following formula:

1 nmol/L = 65 ng/dL

1 ng/dL = 0.0154 nmol/L

**Reference Interval**

The reference range of healthy people is 1.25 – 3.03 nmol/L. It is recommended that each laboratory establish its own reference intervals. The expected values presented here are based on testing of apparently healthy low level samples. Samples were measured in duplicates.

**Sensitivity**

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the guidelines in CLSI document EP17. LoB = 0.22 nmol/L, LoD = 0.32 nmol/L determined with proportions of false positives (α) less than 5 % and false negatives (β) less than 5 %, based on 205 determinations, with 4 blanks and 4 low level samples. LoQ = 0.37 nmol/L, as graphically determined from the precision profile curve.

**Precision**

<table>
<thead>
<tr>
<th>Sample Id</th>
<th>Average nmol/L</th>
<th>Intra-assay CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI</td>
<td>1.90</td>
<td>3.30</td>
</tr>
<tr>
<td>CII</td>
<td>4.49</td>
<td>2.98</td>
</tr>
<tr>
<td>FT4Pool4,l</td>
<td>1.20</td>
<td>3.07</td>
</tr>
<tr>
<td>FT4Pool37,4</td>
<td>3.40</td>
<td>3.44</td>
</tr>
</tbody>
</table>

**Limitations**

- The reagents supplied in this kit are optimized to measure T1- levels in serum.
- Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided.
- Hemolyzed and lipemic specimens may give false values and should not be used.
- The results obtained should only be interpreted in the context of the overall clinical picture. None of the in vitro diagnostic kits can be used as the one and only proof of any disease or disorder.

**Procedural notes**

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmly tightened inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

**Additional information**

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

**Precaution**

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

**Biohazard**

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1) and Hepatitis B surface Antigen (HBsAg).

Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore be handled as potentially infectious materials.