**Description**

The FT$_3$ [¹²⁵I] RIA kit provides a quantitative in vitro determination of free triiodothyronine (FT$_3$) in human serum in the range 0-40 pmol/l (0-26 pg/ml).

**Introduction**

Among the thyroid hormones produced in the thyroid gland triiodothyronin (3,5,3'-triiodo-L-thyronin, T$_3$) is regarded as the most biologically active molecule, produced up to 80 % by the deiodination of tetraiodothyronine (T$_4$) in peripheral tissues. T$_3$ 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. Thyroid disorders are existing only if a net change of binding proteins' concentrations, or thyroid hormone production. Thyroid disorders are existing only if a net change of binding proteins' concentrations, or thyroid hormone production. Thyroid disorders are existing only if a net change of binding proteins' concentrations, or thyroid hormone production. Hyperthyroidism is generally associated with an increase of the FT$_3$ concentration, and in some cases the increased FT$_3$ concentration is the only indicator of T$_3$ thyrotoxicosis. Determination of the free T$_3$ concentration allows also the follow-up of patients under liothyronine therapy.

**Principle of the method**

This assay is based on the competition between FT$_3$ and conjugate (T$_3$ analog bound to biotinylated carrier protein) for a limited number of binding sites on [¹²⁵I]-labelled monoclonal anti-triiodothyronine antibodies (tracer). Allowing to react a fixed amount of conjugate and antibody with different amounts of ligand and the radioactivity measured on the solid phase will be inversely proportional to the concentration of ligand. During a 2-hour incubation period with continuous agitation immuno-complex is immobilized on the reactive surface of test tubes. Decanting the supernatant from all tubes the radioactivity in tubes can be measured in a gamma counter. By plotting binding values against a series of calibrators containing known amount of FT$_3$, a calibration curve is constructed, from which the unknown concentration of FT$_3$ in patient samples can be determined.

**Contents of the kit**

1. FT$_3$ [¹²⁵I] TRACER, ready to use.
2. 1 Pack leaflet.
3. CONJUGATE, ready to use.
4. Tracer, standard and conjugate solutions are available.”

**Materials, tools and equipment required**

Test tube rack, precision pipettes with disposable tips (100 and 500 µl), vortex mixer, shaker, plastic foil, absorbent tissue, Gamma counter

**Recommended tools and equipment for repeating pipettes**

**Preparation of reagents**

Tracer, standard and conjugate solutions are ready to use. Add 500 µl distilled water to the lyophilised human serum in the range 2-6 pmol/l FT$_3$. Ensure that complete dissolution is achieved, and allow the solution to equilibrate at room temperature for at least 20 minutes.

**Specimen collection and storage**

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided. Do not use lipemic, hemolyzed or turbid specimens.

**Assay procedure**

(For a quick guide, refer to Table 1.)

1) Equilibrate reagents and samples to room temperature before use.
2) Label coated tubes in duplicate for total counts (T), zero standard (Standard 1 = B$_0$), standards (S$_2$-S$_6$), control (C) and samples (S$_x$).
3) Homogenize all reagents and samples by gentle mixing to avoid foaming.

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Total (T)</th>
<th>Standard S$_2$-S$_6$</th>
<th>Sample S$_x$</th>
<th>Control (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>500</td>
</tr>
<tr>
<td>Conjugate</td>
<td></td>
<td></td>
<td></td>
<td>500</td>
</tr>
<tr>
<td>Tracer</td>
<td></td>
<td></td>
<td></td>
<td>500</td>
</tr>
</tbody>
</table>

Shake for 2 hours at room temperature.

Decant the fluid and blot on filter paper for 5 minutes.

Count radioactivity (60 sec/testube).

**Calculation of results**

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2. Calculate the average count per minute (CPM) for each pair of assay tubes. Calculate the percent B$_0$/T% for zero standard (S$_0$) by using the following equation:

B$_0$/T% = 100 x S$_1$/T (cpm)/T (cpm)

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:
B/B₀ = 100 x S₂₋₄ ; C : S₄ (cpm)/S₁ (cpm)

For simplicity, these values are uncorrected for non-specific binding (NSB). This is enabled by low NSB being less than 1.5 % of total count.

Using semi-logarithmic graph paper plot B/B₀ (%) for each standard versus the corresponding concentration of FT₃. Figure 1 shows a typical standard curve.

Determine the FT₃ concentration of the unknown samples by interpolation from the standard curve.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

### Table 2. Typical assay data. (Do not use to calculate sample values)

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Counts CPM</th>
<th>Counts CPM2</th>
<th>Mean CPM</th>
<th>B/T %</th>
<th>B/B₀ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>90813</td>
<td>91281</td>
<td>91047</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>44588</td>
<td>45108</td>
<td>44848</td>
<td>49.3</td>
<td>100.0</td>
</tr>
<tr>
<td>S₂</td>
<td>39675</td>
<td>39475</td>
<td>39575</td>
<td>43.5</td>
<td>88.2</td>
</tr>
<tr>
<td>S₃</td>
<td>32476</td>
<td>33349</td>
<td>32913</td>
<td>36.1</td>
<td>73.4</td>
</tr>
<tr>
<td>S₄</td>
<td>26166</td>
<td>26481</td>
<td>26324</td>
<td>28.9</td>
<td>58.7</td>
</tr>
<tr>
<td>S₅</td>
<td>18342</td>
<td>18613</td>
<td>18478</td>
<td>20.3</td>
<td>41.2</td>
</tr>
<tr>
<td>S₆</td>
<td>11149</td>
<td>11142</td>
<td>11281</td>
<td>12.4</td>
<td>25.2</td>
</tr>
<tr>
<td>C</td>
<td>35488</td>
<td>35061</td>
<td>35275</td>
<td>38.7</td>
<td>78.7</td>
</tr>
</tbody>
</table>

### Figure 1. Typical standard curve

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

1 pmol/l = 0.0651 ng/dl

### Characterization of the assay

#### Typical assay parameters

- NSB/T < 1 %
- B₂/T 55±10%
- ED-50 11.4 ±4 pmol/l

#### Specificity

Four analytes were added in different concentrations to T₃ free standard (Sₒ=Bₒ) and the concentration of FT₃ was measured.

### Precision

The within-assay precision was determined with 10 replicates within a single run, both with 5 independent runs carried out in duplicates (using different shakers, range of temperature during incubation 20-30 °C). With 5 samples, CV% values are summarized below.

### Expected Values

It is recommended that each laboratory establishes its own reference intervals. The expected values presented here are based on testing of apparently healthy blood donors. Samples were measured in duplicates. From statistical analysis, the following results were obtained:

### Availability

From stock.

### Shelf life

The minimum shelf life of kit reagents is usually 8 weeks from the date of manufacturing. The actual expiry date is given on the package label and in the quality certificate. To make the maximum benefit of long-term stability it is recommended to adjust the date of ordering to new-batch manufacturing calendar issued each year. Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

### Precautions

#### 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.

### Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 113.5 mg.

### Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (ELIA, 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.

- Used by
- Batch code
- Temperature limitation
- Control
- Caution, consult accompanying documents
- Standard
- Biological risks
- Coated Tube
- Consult instructions for use
- Tracer
- In vitro diagnostic device
- Conjugate
- Manufacturer
- Catalogue number
- Radioactive material

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