RK-339CTACE231031

FT3 [I-125] RIA KIT

(Ref: RK-339CT)

Description

The FT_3 [I-125] RIA system 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, T3) is regarded as the most biologically active molecule, produced up to 80 % by the deiodination of tetraiodothyronine (T4) in pheripheral 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 free unbound fractions occur persistently, therefore the true measure of thyroid status will be the concentration of free hormones.

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 analogue bound to the wall of the test tube) for a limited number of binding sites on ^{125}I-labelled monoclonal anti-triiodothyronine antibodies (tracer). Allowing to react a fixed amount of conjugate and antibody with different amounts of ligand the radioactivity measured on the solid phase will be inversely proportional to the concentration of ligand. During a 1-hour incubation period with continuous agitation immuno-complex is immobilized on the reactive surface of test tubes. Removing 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 bottle	^{125}I-TRACER, ready to use. 22 mL per vial, containing < 260 kBq ^{125}I-labelled monoclonal antibody in buffer with red dye and 0.1 % NaN_3.
6 vials	STANDARDS, ready to use. 1.0 mL per vial, containing approximately 0(S_0), 3 (S_1), 6 (S_2), 12 (S_3), 20 (S_4) and 40 (S_5) pmol/L FT_3 in human serum with 0.1% NaN_3 and 0.5% Kathon CG. *The exact concentrations of standards are listed in the quality certificate enclosed.*
2 vials	CONTROL SERA, ready to use. 1.0 mL per vial, containing human serum with 0.1% NaN_3 and 0.5% Kathon CG. *The concentration of the controls are specified in the quality certificate enclosed.*
2 boxes	COATED TUBE, ready to use. 2X50 reactive test tubes, 12x75 mm. Packed in plastic boxes.
1	Quality certificate
1	Pack leaflet.

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (100, 200 µl), vortex mixer, shaker, plastic foil, absorbent tissue, distilled water, Gamma counter

Recommended tools and equipment

Repeating pipettes (e.g. Eppendorf or else)

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, haemolyzed or turbid specimens.

CAUTION!

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

Use of Control Sera

Good laboratory practices require that control sera be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

Assay procedure

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

1. Label coated tubes in duplicate for each standard (S0-S5), control sera (CI, CII) and samples (U). Optionally, label two test tubes for total counts (T).
2. Homogenize all reagents and samples by gentle mixing to avoid foaming.
3. Pipette **100 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 (min. 600 rpm).
6. Incubate tubes for 1 hour at RT.
7. Add **1.0 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. Count each tube for at least 60 seconds in a gamma counter.
10. Calculate the FT3 concentrations of the samples as described in calculation of results.

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

Tubes	Total	Standard	Control	Sample
Standard		**100**		
Control			**100**	
Sample				**100**
Tracer	**200**	**200**	**200**	**200**
Shake for 1 hour at room temperature				
Distilled water		**1000**	**1000**	**1000**
Remove the water and blot on filter paper for 2 minutes.				
Count radioactivity (60 sec/tube).				
Calculate the results.				

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:

$$\mathbf{B_0/T\ \% = 100 * S_0(cpm) / T\ (cpm)}$$

Calculate the normalized percent binding for ach standard, control and sample respectively by using the following equation:

$$\mathbf{B/B_0\ \% = 100 * S_{1\text{-}5};\ C;\ S_x\ (cpm) / S_0\ (cpm)}$$

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/B_0 (%) for each standard versus the corresponding concentration of FT_3. Figure 1 shows a typical standard curve.

Determine the FT_3 concentration of the unknown samples by interpolation from the standard curve.

Logit-log, or spline curve fit can be selected when using programs applied for computerized data processing.

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

Tubes	Mean cpm	B/T%	B/B_0%
T	117193		
S0	78274	66.8	100.0
S1	62906	53.7	80.4
S2	48612	41.4	62.1
S3	33977	29.0	43.4
S4	20982	17.9	26.8
S5	7733	6.6	9.9
CI	49453	42.2	63.2
CII	38059	32.5	48.6

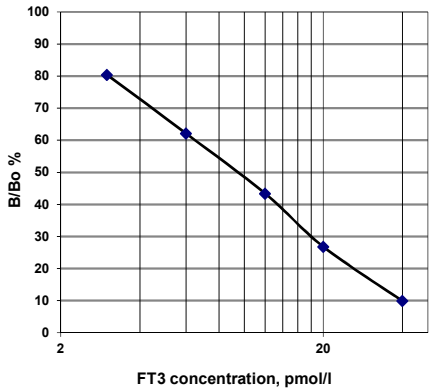

Figure 1.
Typical standard curve

Performance characteristics

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

1 pmol/L = 0.0651 ng/dL

Sensitivity

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the guidelines in CLSI document EP17.

Limit of Blank (LoB): 0.43 pmol/L
Limit of Detection (LoD): 0.78 pmol/L
Limit of Quantitation (LoQ): 1.35 pmol/L

The functional sensitivity is equal to the Limit of Quantitation (LoQ).

Specificity

The specificity of the FT3 [I-125] RIA kit is declared according to the CLSI guide C45-A (5.3.5 Cross-Reactivity)
The specificity of the antibody used in the KIT (Sheep monoclonal antibody to T3, clone T3.17C6) is: T4 cross reactivity < 1 %.

Precision

The within-assay precision was determined with 20 replicates within a single run, the between-assay precision was estimated in 96 independent runs carried out in duplicates. CV values are summarized below.

Intra-assay	
Mean (pmol/L)	CV%
3.24	3.97
3.93	3.43
6.98	2.66
11.77	3.08
18.18	1.33

Inter-assay	
Mean (pmol/L)	CV%
3.22	7.72
7.58	5.41
11.16	4.29
19.37	2.79
24.48	6.23

Reference interval

It is recommended that each laboratory establish its own reference intervals. The expected values presented here are based on testing of apparently healthy blood donors.

A reference range of 3.35 – 7.94 pmol/L has been obtained.

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 firmed tightly 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.

Precautions and warnings

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, 2), Hepatitis-C antibody (anti-HCV), Hepatitis B surface Antigen (HBsAg) and Treponema Antibody. 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 infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.
All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

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 30 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 67 days from availability

	Use by	CONTROL	Control
LOT	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	REF	Catalogue number
IVD	In vitro diagnostic medical device	8°C 2	Store between 2-8°C
	Manufacturer		Radioactive material

CE

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