

FT₃ [¹²⁵I] RIA KIT (Ref:RK-33CT)

Description

The FT₃ [¹²⁵I] RIA system provides a quantitative *in vitro* determination of free triiodothyronine (FT₃) 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 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. 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 FT₃ concentration, and in some cases the increased FT₃ concentration is the only indicator of T₃ thyrotoxicosis.

Determination of the free T₃ concentration allows also the follow-up of patients under liothyronine therapy.

Principle of the method

This assay is based on the competition between FT₃ and conjugate (T₃ 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 the radioactivity measured on the solid phase will be inversely proportional to the concentration of ligand. During a 2hour 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 FT₃, a calibration curve is constructed, from which the unknown concentration of FT₃ in patient samples can be determined.

Contents of the kit

- 1 ¹²⁵I-TRACER, ready to use.
- vial 55 ml per vial, containing about 300 kBq ¹²⁵I-labelled monoclonal antibody in buffer with 0.1 % NaN₃.

- 6 STANDARDS, ready to use.
- vials 0.5 ml per vial, containing 0 (S₁), 2 (S₂), 5 (S₃), 10 (S₄), 20 (S₅) and 40 (S₆) pmol/l FT₃ in human serum with 0.1% NaN₃.

- 1 CONJUGATE, ready to use.
- vial 55 ml per vial, containing conjugate in buffer with 0.1% NaN₃. Do not expose to direct sunlight.

- 1 CONTROL SERUM
- vial Lyophilised human serum with 0.1% NaN₃. The concentration of the control serum is specified in the quality certificate enclosed.

- 2 COATED TUBE, ready to use.
- boxes 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 and 500 µl), vortex mixer, shaker, plastic foil, absorbent tissue
Gamma counter

Recommended tools and equipment
repeating pipettes

Preparation of reagents

Tracer, standard and conjugate solutions are ready to use.
Add 500 µl distilled water to the lyophilised control serum. Mix gently with shaking or vortexing (foaming should be avoided). 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 = B₀), standards (S₂₋₆), control (C) and samples (S_x).
- 3) Homogenize all reagents and samples by gentle mixing to avoid foaming.

- 4) Pipette 100 µl of each standard, control and sample into the properly labelled tubes.
- 5) Pipette 500 µl of conjugate into all tubes except T.
- 6) Pipette 500 µl of tracer solution into all tubes.
- 7) Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube. To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack.
- 8) Incubate tubes for 2 hours at room temperature.
- 9) 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 5 minutes.
- 10) Count each tube for at least 60 seconds in a gamma counter.
- 11) Calculate the FT₃ concentrations of the samples as described in calculation of results.

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

Tubes	Total (T)	Standard (S ₁ -S ₆)	Sample (S _x)	Control (C)
Standard		100		
Sample			100	
Control				100
Conjugate		500	500	500
Tracer	500	500	500	500
Shake for 2 hours at room temperature.				
Decant the fluid and blot on filter paper for 5 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 B₀/T% for zero standard (S₁) by using the following equation:
B₀/T % = 100 * S₁(cpm) / T (cpm)
Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/B_0 \% = 100 * S_{2-6} / C ; S_x \text{ (cpm)} / S_1 \text{ (cpm)}$$

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)

Tubes	Counts CPM1	Counts CPM2	Mean CPM	B/T %	B/Bo %
T	90813	91281	91047		
S1	44588	45108	44848	49.3	100.0
S2	39675	39475	39575	43.5	88.2
S3	32476	33349	32913	36.1	73.4
S4	26166	26481	26324	28.9	58.7
S5	18342	18613	18478	20.3	41.2
S6	11419	11142	11281	12.4	25.2
C	35488	35061	35275	38.7	78.7

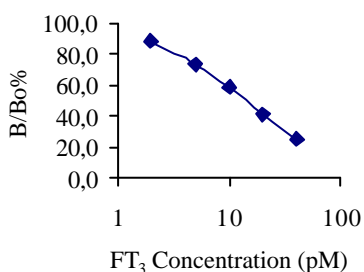


Figure 1.

Typical standard curve

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

$$1 \text{ pmol/l} = 0.0651 \text{ ng/dl}$$

Characterization of the assay

Typical assay parameters

NSB/T	<1 %
B ₀ /T	55±10%
ED-50	11.4 ±4 pmol/l

Specificity

Four analytes were added in different concentrations to T₃ free standard (S₁=B₀) and the concentration of FT₃ was measured.

Analyte added	Conc. (nmol/l)	FT ₃ measured (pmol/l)
r-T ₃	100	<DL
r-T ₃	1000	3.1
r-T ₃	10000	28.6
3,3' diiodo-L-thyronine	3	1.3
3,3' diiodo-L-thyronine	10	4.2
3,3' diiodo-L-thyronine	30	16.1
3,5 diiodo-L-thyronine	10	2.1
3,5 diiodo-L-thyronine	30	4.6
3,5 diiodo-L-thyronine	300	38.3
T ₄	79	<DL
T ₄	258	1.19
T ₄	412	3.28

DL – detection limit

Sensitivity

Better than 0.58 pmol/l, corresponding to the 0-2xSD value.

Precision

The within-assay precision was determined with 10 replicates within a single run, the between-assay precision was estimated in 13 independent runs carried out in duplicates (using different shakers, range of temperature during incubation 20-30 °C) , both with 5 samples. CV% values are summarized below.

Intra-assay		Inter-assay	
Mean (pmol/l)	CV %	Mean (pmol/l)	CV %
2.43	9.0	2.71	12.4
3.39	4.6	3.45	7.98
6.44	3.3	6.51	4.76
11.6	3.2	12.9	8.04
40.0	2.2	39.2	5.62

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:

Age (years)						
	n	Mean	SD	Min	Max	BL
Female	197	35.4	12.1	18	63	-
Male	200	35.4	11.7	18	64	-
Male+female	397	35.4	11.9	18	64	-
FT ₃ (pmol/l)						
Female	197	3.35	0.80	1.9	10.2	1.7-5.0
Male	200	3.95	0.63	2.4	6.6	2.7-5.2
Male+female	397	3.65	0.78	1.9	10.2	2.5-5.4

BL= Borderline for upper and lower 2.5 % from distribution.

As a guide (mean ± 2*SD), 2.09–5.21 pmol/l reference range was obtained from normal patients based on statistical consideration only. Taking into consideration not only statistical results but clinical practice as well more realistic **reference range can be recommended 1.9-5.7 pmol/l.**

Additional information

Storage

Store the reagents between 2-8°C. At this temperature each reagent is stable until expiry date. Control serum should be aliquoted and stored deep frozen (-20°C) for a repeated use.

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 (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*.

 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

REF Catalogue number

 Radioactive material

CE

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