RK-34CTACE040501

FT_4 [^{125}I] RIA KIT

(Ref:RK-34CT)

Description

The FT_4 [^{125}I] RIA system provides a quantitative in vitro determination of free thyroxine (FT_4) in human serum. Using 50 µl serum sample FT_4 can be assayed in the range 0-100 pmol/l (0-7.77 ng/dl). Each kit contains materials sufficient for 100 assay tubes permitting the construction of one standard curve and the assay of 42 unknowns in duplicate.

Introduction

Circulating thyroid hormones (thyroxin, T_4 and triiodothyronine, T_3) are distributed into two, a major, protein-bound, and a minor, (0.03 % of T_4 and 0.3 % of T_3) free, compartments. 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 clinical utility of total T_4 and T_3 is dependant on the knowledge of functional levels of binding proteins.

Serum level of free T_4 (FT_4) correlates very well with secretion and metabolism rate of T_4, and has been recommended as the most reliable and meaningful diagnostic indicator of thyroid diseases, mostly in conflicting or borderline instances. Apparent FT_4 levels, however, are very sensitive to the analytical method due to the sophisticated multiple equilibrium between various protein compartments of T_4.

Principle of the method

This assay is based on the competition between FT_4 and conjugate (T_4 analog bound to biotinylated carrier protein) for a limited number of binding sites on ^{125}I-labelled monoclonal antithyroxine 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 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_4, a calibration curve is constructed, from which the unknown concentration of FT_4 in patient samples can be determined.

Contents of the kit

1 vial	^{125}I-TRACER, ready to use. 55 ml per vial, containing about 260 kBq ^{125}I-labelled monoclonal antibody in buffer with 0.1 % NaN_3.
6 vials	STANDARDS, ready to use. 0.5 ml per vial, containing 0 (S_1), 6 (S_2), 12 (S_3), 25 (S_4), 50 (S_5) and 100 (S_6) pmol/l FT_4 in human serum with 0.1% NaN_3.
1 vial	CONJUGATE, ready to use. 55 ml per vial, containing conjugate in buffer with 0.1% NaN_3. Do not expose to direct sunlight.
1 vial	CONTROL SERUM Lyophilised human serum with 0.1% NaN_3. The concentration of the control serum is 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 (50 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 = B_0), standards (S_{2-6}), control (C) and samples (S_x). *(See Note-1)*

3) Homogenize all reagents and samples by gentle mixing to avoid foaming.

4) Pipette 50 µl each of standards, control and samples 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. *(See Note-2)*

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 FT_4 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 (M_x)	Control (C)
Standard		50		
Sample			50	
Control				50
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 B_0/T% for zero standard (S_1) by using the following equation:

B_0/T % =100* S_1(cpm)/ T (cpm)

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

B/B_0 %=100* S_{2-6} ; C ; M_x (cpm)/ S_1 (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_4. Figure 1 shows a typical standard curve.

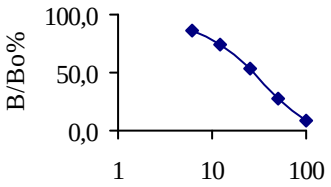
Determine the FT_4 concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

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

Table 2. Typical assay data

Tubes	Counts CPM1	Counts CPM2	AVG CPM	B/T %	B/Bo %
T	97490	97012	97251		
S1	65786	66455	66121	68,0	100,0
S2	57461	56841	57151	58,8	86,4
S3	49265	47834	48550	49,9	73,4
S4	35149	35469	35309	36,3	53,4
S5	18395	18194	18295	18,8	27,7
S6	5695	5690	5693	5,9	8,6
C	44652	44275	44464	45,7	67,2

Figure 1.
Typical standard curve
(Do not use to calculate sample values)



Conversion of SI units can be performed according to the following formula:
1 pmol/l = 0.0777 ng/dl

Characterization of the assay

Typical assay parameters

NSB/T	< 1.5 %
B_0/T	69 ± 9 %
ED-50	27 ± 6 pmol/l

Specificity

T_3 and r-T_3 were added in 4 different concentrations to T_4 free standard (S_1=B_0) and the concentration of FT_4 was measured. The cross reactivity are shown below.

T_3 added (nmol/l)	FT_4 measured (pmol/l)	r-T_3 added (nmol/l)	FT_4 measured (pmol/l)
1	<DL	1	<DL
10	0,8	10	0,9
100	6,3	100	1,7
500	32,7	500	9,1
1000	57,8	1000	18,6

DL – detection limit

Sensitivity

Better than 0.7 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 8 independent runs carried out in duplicates, both with 7 samples. CV values are summarized below.

	Intra-assay		Inter-assay	
	Mean (pmol/l)	CV %	Mean (pool/l)	CV %
1	4,9	7,3	4,9	10,2
2	8,42	3,47	9,15	5,57
3	13	1,49	14	2,83
4	15,8	1,28	15,5	6,63
5	20,2	1,74	22,8	3,58
6	31,5	0,94	39,9	7,02
7	57,8	2,05	70,7	3,89

Expected Values

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. Samples were measured in duplicates unseeing different kit lots.

In a population (n=243) of adult female blood donors (ages: mean 37,8 ± 11.3, range 19 - 69) serum concentrations of FT_4 were 14,26 ± 1.91 pmol/l (mean ± SD). Sample values were found scattered in a range of 10.1 – 22 pmol/l.

In a population (n=243) of adult male blood donors (ages: mean 29.0 ± 10.5 range 19 - 61) serum concentrations of FT_4 were 15.4 ± 2,32 pmol/l (mean ± SD). Sample values were found scattered in a range of 10.1 – 22,5 pmol/l.

For female and male (n=486, ages: mean 33.4 ± 11,7, range 19 - 69) the serum concentration of FT_4 was 14.83 ± 2,2 pmol/l (mean ± SD), range 10.1 – 22,5 pmol/l.

As a guide **(mean ± 2*SD), 10.4 – 19,2** 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 **10-22 pmol/l.**

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.

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

Storage

Store the reagents between 2-8°C. At this temperature each reagent is stable until expiry date. Control serum should be aliquotted 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	LOT	Batch code
	Temperature limitation	CONTROL	Control
	Caution, consult accompanying documents	CAL	Standard
	Biological risks	CT	Coated Tube
	Consult instructions for use	TRAC	Tracer
IVD	*In vitro* diagnostic device	CONJ	Conjugate
	Manufacturer		
REF	Catalogue number		
	Radioactive material		

CE

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