RK-609CTACE161101

T3 [I-125] RIA KIT

(REF: RK-609CT)

The T_3 [I-125] RIA system provides a quantitative *in vitro* determination of L-3,5,3'-triiodothyronine (T_3) 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 up to 80 % by the deiodination of tetraiodothyronine (T4) in pheripheral 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 conjuction with other tests. T3 alone cannot diagnose hypothyroidism, but it may be more sensitive than T4 for hyperthyroidism.

Principle of method

This assay is based on the competition between unlabelled T_3 and fixed quantity of ^{125}I-labelled T_3 for limited number of binding sites on T_3 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 a series of calibrators containing known amount of T_3, a calibration curve is constructed, from which the unknown concentration of T_3 in patient samples can determined.

Contents of the kit

1. 1 bottle ^{125}I-TRACER (22 mL), ^{125}I-labelled T_3 in buffer with red dye and 0.1 % NaN_3, Kathon CG, containing about <260 kBq.

2. 6 vials STANDARD (6 x 1.0 mL), containing (S0-S5) T_3 in serum with 0.1% NaN_3 and Kathon CG. Standard sera nominal values are: 0; 0,75; 1,5; 3; 6; 12 nmol/L. The actual concentration values of the standards can be found in the *Quality certificate sheet enclosed.*

3. 2 vials CONTROL SERUM, 2 x 1.0 mL serum with 0.1% NaN_3 and Kathon CG.

The concentration of the control serum is specified in the *Quality certificate sheet enclosed.*

4. 2 boxes COATED TUBE, 2x50 pcs, 12x75 mm packed in plastic boxes.

Quality certificate

Pack leaflet with instructions for use (IFU)

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (50, 200 and 1000 µL), shaker, plastic foil, absorbent tissue, gamma counter, distilled water.

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.

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

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

1. Equilibrate reagents and samples to room temperature (RT) before use (min. for an hour).
2. 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).
3. Homogenize all reagents and samples by gentle mixing to avoid foaming.
4. Pipette 50 µl of each standard, control and samples into the properly labelled coated tubes.
5. Pipette 200 µl of tracer into each tube.
6. 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.
7. Incubate tubes for 1 hour at RT.
8. Add 1 mL distilled water to each tube.
9. 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.
10. Count each tube for at least 60 seconds in a gamma counter.
11. Calculate the T_3 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.

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:

$$B_0/T\% = \frac{S_0\ (cpm)}{T\ (cpm)} \times 100$$

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

$$B/B_0(\%) = \frac{S_{1\text{-}5}/\ CI,\ CII\ /\ U_x\ (cpm)}{S_0\ (cpm)} \times 100$$

Using semi-logarithmic graph paper plot B/B_0 (%) for each standard versus the corresponding concentration of T_3. Figure 1. shows a typical standard curve. Determine the T_3 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*)

	T	S_0-S_5	CI/CII	U_x
Standard		**50**		
Control			**50**	
Samples				**50**
Tracer	**200**	**200**	**200**	**200**
* Shake for 1 hour at room temperature				
Distilled water		**1000**	**1000**	**1000**
Decant the fluid and blot on filter paper (2 minutes)				
Count radioactivity (60 sec/tube)				
Calculate the results				

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

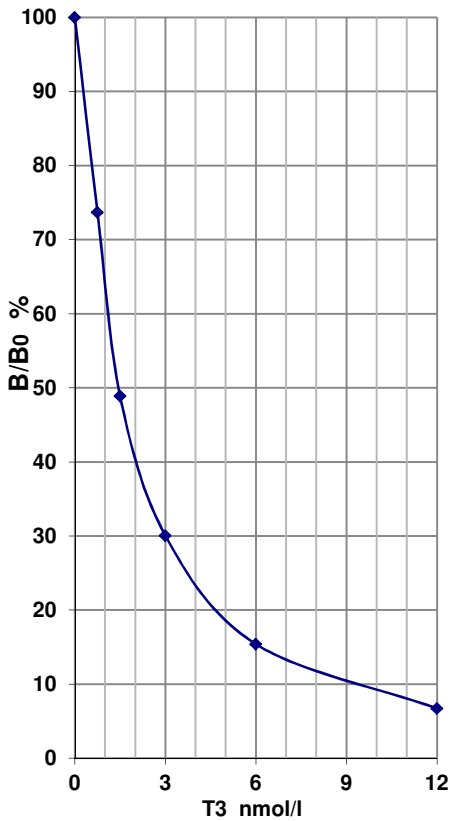


Figure 1.
A typical standard curve
(Do not use to calculate sample values!)

Table 2. Typical assay data

Tubes	Count cpm	Mean cpm	B_0/T %	B/B_0 %
T	117 173 116 098	116 636		
S0	91 002 90 977	90 990	78.5	100.0
S1	66 959 67 119	67 039		73.7
S2	44 397 44 591	44 494		48.9
S3	27 296 27410	27 353		30.1
S	14 000 14 050	14 025		15.4
S5	5 922 6 326	6 124		6.7
CI	40 186 40 184	40 185		44.2
CII	21 628 21 000	21 314		23.4

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 blood donors. Samples were measured in duplicates.

Sensitivity

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the guidlines 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 (ß) 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

Intra-assay precision table

Sample Id	Average nmol/L	Intra-assay CV%
CI	1.90	3.30
CII	4.49	2.98
FT4Pool4,1	1.20	3.07
FT4Pool37,4	3.40	3.44

Inter-assay precision table

Sample ID	Average nmol/L	InterAssay CV%
RK-6CT_1	0.22	23.9
T3Pool-A	0.67	8.6
T3Pool-B	1.16	6.3
T3Pool-C	2.13	4.5
T3Pool-E	3.91	4.1
FT4Pool63.9	7.20	4.1

Recovery

Recovery is defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of T3. The mean (±SD) recovery % for added IRMM-469 T3 (6 samples, 3, 5, 8 nM added T3) was 76.8 ± 7.2 %

Linearity

The linearity of the method was tested using 4 different concentration samples at 2x, 4x and 8x dilution. Dilution using KIT's zero standard as diluent gave an average of 117 % dilution-recovery.

Specificity

Crossreactivity for L-thyroxine T4 were extremely low, undetectable.

Limitations

•The reagents supplied in this kit are optimized to measure T_3 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 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.

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).

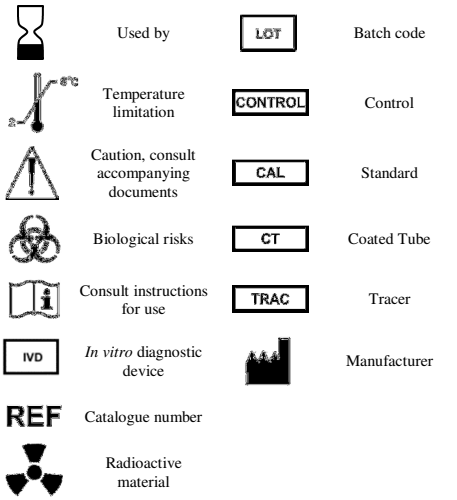
Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore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

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.
The total Kathon CG 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.



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