RK-6CT1ACE040501

T_3 [^{125}I] RIA KIT

(REF: RK-6CT1)

The T_3 [^{125}I] 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 (T_3) is regarded as the most biologically active molecule, produced up to 80 % by the deiodination of tetraiodothyronine (T_4) 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.

T_3 contributes significantly to the maintenance of the euthyroid state, and the total T_3 level has a role in screening for thyroid disease in conjuction with other tests. T_3 alone cannot diagnose hypothyroidism, but it may be more sensitive than T_4 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 2-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 vial ^{125}I-TRACER (11 ml), ^{125}I-labelled T_3 in buffer with red dye and 0.1 % NaN_3, containing about <260 kBq.

2. 1 bottle ANTISERUM (105 ml), containing anti-T_3 IgG in buffer with blue dye and 0.1 % thimerosal.

3. 6 vials STANDARD (6 x 0.5 ml), containing (S1-S6) 0; 0,5; 1,5; 3; 6; 12 nmol/l T_3 in human serum with 0.1% NaN_3.

4. 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.

5. 2 boxes COATED TUBE, 2x50 pcs, 12x75 mm packed in plastic boxes.

Quality certificate
Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (100 and 1000 µl), shaker, plastic foil, absorbent tissue, 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, hemolyzed or turbid specimens.

Preparation of reagents, storage

Store the reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date. The actual expiry date is given on the package label and in the quality certificate.
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. Store at 2-8°C until expiry date.

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 before use (min. for an hour).
2. Label coated tubes in duplicate for each standard (S1-S6), control serum (C) and samples (M). Optionally, label two test tubes for total count (T).
3. Homogenize all reagents and samples by gentle mixing to avoid foaming.
4. Pipette 100 µl each of standards, control and samples into the properly labelled tubes.
5. Pipette 100 µl of tracer into each tube.
6. Pipette 1000 µl of antiserum into each tube except T.
7. 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 shaking in each tube.
8. Incubate tubes for 2 hours at room temperature.
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:

$$B_0/T\% = \frac{S_1\ (cpm)}{T\ (cpm)} \times 100$$

B_0/T% is an optional quality control parameter unnecessary for determination of sample concentrations.
Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/B_0(\%) = \frac{S_{2\text{-}6} / C / M_x\ (cpm)}{S_1(cpm)} \times 100$$

For simplicity, these values are uncorrected for non-specific binding (NSB). This is enabled by low NSB being less than 3 % of total count.
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 can be used.

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

	T	S1-S6	C	M
Standard		**100**		
Control			**100**	
Samples				**100**
Tracer	**100**	**100**	**100**	**100**
Antiserum		**1000**	**1000**	**1000**
Shake for 2 hours at room temperature				
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

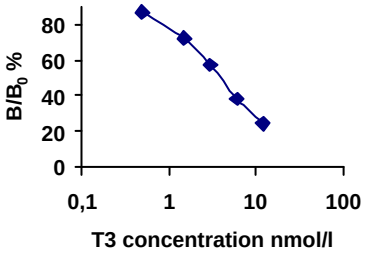

Figure 1.
A typical standard curve
(Do not use to calculate sample values!)

Table 2. Typical assay data

Tubes	Count cpm	Mean cpm	B0/T %	B/B0 %
T	82800 82414	82607		
S1	41973 40338	41156	49.8	100.0
S2	35009 36386	35698	43.2	86.7
S3	29766 29650	29708	36.0	72.2
S4	23583 23702	23643	28.6	57.4
S5	15502 16145	15824	19.2	38.4
S6	9973 10191	10082	12.2	24.5
C	28840 28936	28888	35.0	70.2

Characterization of assay

Typical assay parameters

B_0/T = 45 ± 6 %
ED-80 = 4.5 ± 0.9 nmol/l

Specificity
Cross reactivity values are shown below.

3,5,3'-L-triiodothyronine (T_3)	100 %
Thyroxine (T_4)	<0.06 %
3',5',3,-triodo-L-thyronine (rT_3)	<0.016 %
3,3'-diiodo-L-thyronine (3,3'-T_2)	<1.9 %

Sensitivity
Better than 0.3 nmol/l, corresponding to the 0-2xSD value.

Precision
5 control samples were assayed in 10 replicates to determine intra-assay precision. Values obtained are shown below.

Sample	Mean value nmol/l	SD nmol/l	CV %
1	1.83	0.11	5.8
2	2.06	0.12	5.9
3	2.29	0.10	4.5
4	3.10	0.19	6.0
5	9.32	0.46	5.0

Reproducibility
To determine inter-assay precision 5 control samples were measured in duplicates in 7 independent assays. Values obtained are shown below.

Sample	Mean value nmol/l	SD nmol/l	CV %
1	0.91	0.10	10.6
2	1.72	0.07	4.3
3	1.76	0.08	4.6
4	2.37	0.10	4.0
5	4.31	0.17	3.9

Recovery
Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of T_3. The mean (±SD) recovery % for added T_3 (5 samples, 2.5 nM added T_3) was 102.1 ± 3.9.

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.
In a population (n=120) of adult female blood donors serum concentrations of T_3 were 2.18 ± 0.5 (as mean ± SD). Sample values were found scattered in a range of (1.25 - 3.91). As a guide, 1.4 - 3.3 nmol/l (91 – 215 ng/dl) can be interpreted as reference range for normal patients.
In a population (n=118) of adult male blood donors serum concentrations of T_3 were 1.80 ± 0.38 (as mean ± SD). Sample values were found scattered in a range of (0.55 – 2.75). As a guide, 1.0 – 2.6 nmol/l (65 – 169 ng/dl) can be interpreted as reference range for normal patients.
For female and male (n=238) the mean (±SD) serum concentration of T_3 was 1.99 ± 0.49 in a range (0.55 - 3.91). As a guide, 1.0 - 3.3 nmol/l (65 – 214.5 ng/dl) reference range was obtained from normal patients.
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.

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

1 nmol/l = 0.65 ng/ml
1 µg/dl = 1.54 nmol/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. 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 14.5 mg.


Used by


Temperature limitation


Caution, consult accompanying documents


Biological risks


Consult instructions for use

In vitro diagnostic device


Manufacturer

REF Catalogue number


Radioactive material

LOT Batch code

CONTROL Control

CAL Standard

CT Coated Tube

TRAC Tracer

AS Antiserum

CE

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