T4-RIA-CT

KIR1641

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B-1348 Louvain-la-Neuve - Belgium

History

Resume of change :

Previous Version : 140522/1	**Current Version :** 190711/1
CONTROL | N Controls - N = 1 or 2 in human serum with gentamycin and thymol	CONTROL | N Controls - N = 1 or 2 in human plasma with thymol
LOT : 140522/1	**Version :** 190711/1
No history	History added

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Read entire protocol before use.

T4-RIA-CT

I. INTENDED USE

Radioimmunoassay for the determination of human Thyroxine (T4) in serum. For research use only, not for use in diagnostic procedures.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource T4-RIA-CT Kit

B. Catalog number : KIR1641 : 96 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

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Tel : 1-888-523-1246 Fax : 1-763-780-2988 Email : info@ibl-america.com

III. BACKGROUND

A. Biological activity

L-Thyroxine (T4) is a hormone that is synthesized and stored in the thyroid gland. Proteolytic cleavage of follicular thyroglobulin releases T4 into the bloodstream. Greater than 99% of T4 is reversibly bound to three plasma proteins in blood – thyroxine binding globulin (TGB) binds 70%, thyroxine binding pre-albumin (TBA) binds 20%, and albumin binds 10%. Approximately 0.03% of T4 is in the free, unbound state in blood at any one time.

B. Applications

Diseases affecting thyroid function may present a wide array of confusing symptoms. Determination of total T4 by immunoassay is the most reliable and convenient screening test available to determine the presence of thyroid disorders in research subjects. Increased levels of T4 have been found in hyper-thyroidism due to Grave's disease and Plummer's disease and in acute and sub acute thyroiditis. Low levels of T4 have been associated with congenital hypothyroidism, myxedema, chronic thyroiditis (Hashimoto's disease) and with some genetic abnormalities.

IV. PRINCIPLES OF THE METHOD

A fixed amount of ^{125}I labelled T4 competes with the T4 to be measured present in the sample or in the calibrator for a fixed amount of anti-T4 antibody sites, which are bound to the goat anti mouse (GAM) antibodies immobilized to the wall of a polystyrene tube. After 1 hour incubation at room temperature, an aspiration step terminates the competition reaction. The tubes are then washed with 2 ml of working wash solution and aspirated again. A calibration curve is plotted and the T4 concentrations of the samples are determined by dose interpolation from the calibration curve.

V. REAGENTS PROVIDED

Reagents	96 Test Kit	Colour Code	Reconstitution
Tubes coated with GAM (Goat anti Mouse)	2 x 48	black	**Ready** for use
Ag | **^{125}I** TRACER: ^{125}Iodine labelled T4 (HPLC grade) in phosphate buffer with bovine casein and azide (<0.1%)	1 vial 21 ml 111 kBq	red	**Ready** for use
CAL | **0** Zero Calibrator in human serum, gentamycin and thymol	1 vial lyophil.	yellow	**Add** 0.5 ml distilled water
CAL | **N** Calibrators - N = 1 to 5 (see exact values on vial labels) in human serum, gentamycin and thymol	5 vials lyophil.	yellow	**Add** 0.5 ml distilled water
Ab Anti-T4 (monoclonal) antibodies in phosphate buffer with bovine serum albumin and thymol	1 vial lyophil.	blue	**Add** 11ml distilled water
WASH | **SOLN** | **CONC** Wash solution (TRIS-HCl)	1 vial 10 ml	brown	**Dilute** 70 x with distilled water (use a magnetic stirrer).
CONTROL | **N** Controls - N = 1 or 2 in human plasma with thymol	2 vials lyophil.	silver	**Add** 0.5 ml distilled water

Note : Use the zero calibrator for sera dilutions.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Distilled water
2. Pipettes for delivery of: 20 µl, 100 µl, 200 µl and 500 µl (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Tube shaker
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional)
8. Any gamma counter capable of measuring ^{125}I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

A. Calibrators: Reconstitute the calibrators with 0.5 ml distilled water.
B. Controls: Reconstitute the controls with 0.5 ml distilled water.
C. Anti-T4: Reconstitute the anti-T4 with 11 ml distilled water.
D. Working Wash solution: Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators and controls are stable for 7 days at 2-8°C. For longer storage periods, aliquots should be made and kept at –20°C for maximum 3 months. Avoid subsequent freeze-thaw cycles.
- After reconstitution, the anti-T4 antibodies are stable for 6 weeks at 2-8°C. **DO NOT FREEZE.**
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- Serum samples must be kept at 2-8°C.
- If the test is not run within 24 hrs, storage at -20°C is recommended.
- Avoid subsequent freeze-thaw cycles.

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiry date.
Do not mix materials from different kit lots.
Bring all the reagents to room temperature prior to use.
Thoroughly mix all reagents and samples by gentle agitation or swirling.
Use a clean disposable pipette tip for addition of each different reagent and sample in order to avoid cross-contamination. High precision pipettes or automated pipetting equipment will improve the precision.
Respect the incubation times.
Prepare a calibration curve for each run, do not use data from previous runs.

B. Procedure

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes
2. Briefly vortex calibrators, controls and samples and dispense 20µl of each into the respective tubes.
3. Dispense 200 µl of ^{125}Iodine labelled T4 into each tube, including the uncoated tubes for total counts.
4. Dispense 100 µl of anti-T4 into each tube, except tubes for total counts.
5. Shake the tube rack gently by hand to liberate any trapped air bubbles.
6. Incubate for 1 hour at room temperature with continous shaking.
7. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
8. Wash tubes with 2 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
9. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
10. Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula :

$$B/B0\ (\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

3. Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B0(%)) values for each calibrator point as a function of the T4 concentration of each calibrator point. Reject obvious outliers.
4. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
5. By interpolation of the sample (B/B0 (%)) values, determine the T4 concentrations of the samples from the calibration curve.
6. For each assay, the percentage of total tracer bound in the absence of unlabelled T4 (B0/T) must be checked.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

T4		cpm	B/Bo (%)
Total count		32598	B/B0
Calibrator	0 nmol/l	12661	100.0
	12.8 nmol/l	11434	90.3
	32 nmol/l	8460	66.8
	80 nmol/l	4554	36.0
	200 nmol/l	2020	16.0
	500 nmol/l	932	7.4

XIII. PERFORMANCE AND LIMITATIONS

A. Detection limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was < 5 nmol/l.

B. Specificity

The percentage of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively:

Compound	Cross-Reactivity (%)
L- thyoxine (L-T4)	100
D-thyroxine (D-T4)	48
L-3,3',5 - triiodothyronine (L-T3)	1.01
L-3,3',5' - triiodothyronine (rT3)	7

Note: this table shows the cross-reactivity for the anti T4

C. Precision

INTRA-ASSAY PRECISION

Serum	N	<X> ± SD (nmol/l)	CV (%)
A	10	32.4 ± 1.8	5.6
B	10	183.8 ± 5.9	3.2

INTER-ASSAY PRECISION

Serum	N	<X> ± SD (nmol/l)	CV (%)
A	18	32.7 ± 2.1	6.5
B	20	235.3 ± 15.2	6.5

SD: Standard Deviation; CV: Coefficient of variation

D. Accuracy

DILUTION TEST

Sample	Dilution	Theoretical Concent. (nmol/l)	Measured Concent. (nmol/l)
A	1/1	-	425
	1/2	212.5	194.3
	1/4	106.3	89.1
	1/8	53.1	48.8
	1/16	26.6	22.5

Samples were diluted with zero calibrator.

RECOVERY TEST

Sample	added T4 (nmol/l)	Recovered T4 (nmol/l)	Recovered (%)
1	32.2	27.1	84%
	64.4	67.8	105%
	128.7	132.6	103%
	257.4	290.5	113%
	386.1	444.8	115%

To the best of our knowledge, no international reference material exists for this parameter.

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 60 minutes after the calibrator has been added to coated tubes.

TIME DELAY

Serum nmol/l	0'	20'	40'	60'
C 1	46.5	43.0	38.6	36.4
C 2	202.5	212.6	207.3	201.0

XIV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.

XV. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.
T4 concentrations for untreated euthyroid subjects (n=298) ranged from 60 to 157 nmol/l . The ranges are expressed as 2.5% to 97.5% percentiles

XVI. PRECAUTIONS AND WARNINGS

Safety

For research use only, not for use in diagnostic procedures.
This kit contains ^{125}I (half-life: 60 days) ,emitting ionizing X (28 keV) and γ (35.5 keV) radiations. This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.
All radioactive handling should be executed in a designated area. away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.
Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.
The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HbsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.
All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.
Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

XVII. BIBLIOGRAPHY

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XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS µl	**CALIBRATORS µl**	**SAMPLE (S) CONTROLS µl**
Calibrators (0 to 5) Samples, Controls Tracer Anti-T4	- - 200 -	20 - 200 100	- 20 200 100
Incubation	1 hour at room temperature with continuous shaking		
Separation Working Wash solution Separation	-	Aspirate (or decant) 2.0 ml Aspirate (or decant)	
Counting	Count tubes for 60 seconds		

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