

Read entire protocol before use.

## T3-RIA-CT

### I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of human 3,5,3' Triiodothyronine (T3) in serum.

### II. GENERAL INFORMATION

- A. **Proprietary name :** DIAsource T3-RIA-CT Kit
- B. **Catalog number :** KIP1631 : 96 tests  
KIP1634: 4 x 96 tests
- C. **Manufactured by :** DIAsource ImmunoAssays S.A.  
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

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### III. CLINICAL BACKGROUND

#### A. Biological activity

The thyroid gland exerts powerful and essential regulatory influences on growth, differentiation, cellular metabolism, and general hormonal balance, as well as on the maintenance of metabolic activity and the development of the skeletal and organ system.

The hormones thyroxine (T4) and 3,5,3' triiodothyronine (T3) circulate in the blood stream, mostly bound to the plasma protein, thyroxine binding globulin (TBG). The concentration of T3 is much less than that of T4, but its metabolic potency is much greater.

#### B. Clinical applications

T3 determination is an important factor in the diagnosis of thyroid disease. Its measurement has uncovered a variant of hyperthyroidism in thyrotoxic patient with elevated T3 levels and normal T4 levels. An increase in T3 without an increase in T4 is frequently a forerunner of recurrent thyrotoxicosis in previously treated patients. In other patients, euthyroidism is attributable to normal T3, although their T4 values are subnormal.


T3 determination is also useful in monitoring both patient under treatment for hyperthyroidism and patients who have discontinued anti-thyroid drug therapy. It is especially valuable in distinguishing between euthyroid subjects.

In women, T3 levels are elevated during pregnancy, during estrogen treatment, and contraceptive hormone therapy. When T3 levels parallel TBG increases in a manner analogous to T4 levels, these changes are not a reflection of altered thyroid status.

#### IV. PRINCIPLES OF THE METHOD

A fixed amount of <sup>125</sup>I labelled T3 competes with the T3 to be measured present in the sample or in the calibrator for a fixed amount of anti-T3 antibody sites, which are bound to the goat anti mouse 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 T3 concentrations of the samples are determined by dose interpolation from the calibration curve.

#### V. REAGENTS PROVIDED

Reagents	96 Test Kit	4 x 96 Test Kit	Colour Code	Reconstitution			
 Tubes coated with GAM (Goat anti Mouse)	2 x 48	8 x 48	black	Ready for use			
<table border="1" data-bbox="76 600 215 638"><tr><td>Ag</td><td>125I</td></tr></table> TRACER: <sup>125</sup> Iodine labelled T3 (HPLC grade) in phosphate buffer with bovine casein and azide (<0.1%)	Ag	125I	1 vial 21 ml 111 kBq	4 vials 21 ml 4x111 kBq	red	Ready for use	
Ag	125I						
<table border="1" data-bbox="76 750 215 788"><tr><td>CAL</td><td>0</td></tr></table> Zero Calibrator in human serum and thymol	CAL	0	1 vial lyophil.	2 vials lyophil.	yellow	Add 0.5 ml distilled water	
CAL	0						
<table border="1" data-bbox="76 862 215 900"><tr><td>CAL</td><td>N</td></tr></table> Calibrators - N = 1 to 5 (see exact values on vial labels) in human serum and thymol	CAL	N	5 vials lyophil.	10 vials lyophil.	yellow	Add 0.5 ml distilled water	
CAL	N						
<table border="1" data-bbox="76 990 167 1028"><tr><td>Ab</td></tr></table> Anti-T3 (monoclonal) antibodies in phosphate buffer with bovine serum albumin and thymol	Ab	1 vial lyophil.	4 vials lyophil.	blue	Add 11ml distilled water		
Ab							
<table border="1" data-bbox="76 1131 263 1169"><tr><td>WASH</td><td>SOLN</td><td>CONC</td></tr></table> Wash solution (TRIS-HCl)	WASH	SOLN	CONC	1 vial 10 ml	4 vials 10 ml	brown	Dilute 70 x with distilled water (use a magnetic stirrer).
WASH	SOLN	CONC					
<table border="1" data-bbox="76 1243 236 1281"><tr><td>CONTROL</td><td>N</td></tr></table> Controls - N = 1 or 2 in human serum with thymol	CONTROL	N	2 vials lyophil.	4 vials lyophil.	silver	Add 0.5 ml distilled water	
CONTROL	N						

**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: 50 µl, 100 µl, 200 µl, 500 µl and 2 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Tube shaker (700 rpm)
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional)
8. Any gamma counter capable of measuring <sup>125</sup>I may be used (minimal yield 70%).

#### VII. REAGENT PREPARATION

- Calibrators:** Reconstitute the zero calibrator with 0.5 ml distilled water and the other calibrators with 0.5 ml distilled water.
- Controls:** Reconstitute the controls with 0.5 ml distilled water.
- Anti-T3:** Reconstitute the anti-T3 with 11 ml distilled water.
- 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-T3 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 50µl of each into the respective tubes.
3. Dispense 200 µl of <sup>125</sup>Iodine labelled T3 into each tube, including the uncoated tubes for total counts.
4. Dispense 100 µl of anti-T3 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 continuous 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 :

$$B/B_0(\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

3. Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B<sub>0</sub>(%)) values for each calibrator point as a function of the T3 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/B<sub>0</sub> (%)) values, determine the T3 concentrations of the samples from the calibration curve.
6. For each assay, the percentage of total tracer bound in the absence of unlabelled T3 (B<sub>0</sub>/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.

T3	cpm	B/Bo (%)
Total count	40019	
Calibrator		
0.00 nmol/l	28572	100.0
0.35 nmol/l	24781	86.7
1.00 nmol/l	18112	63.4
2.50 nmol/l	10587	37.1
6.50 nmol/l	4629	16.2
14.00 nmol/l	2684	9.4

## 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 0.1 nmol/l.

### B. Specificity

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

Compound	Cross-Reactivity (%)
L-3,3',5 - triiodothyronine (L-T3)	100
3,3',5' - triiodothyronine (rT3)	ND
L- thyroxine (L-T4)	0.17
D-thyroxine (D-T4)	0.04
3,3',5 - triiodothyroacetic acid (TRIAC)	52
3,5 - diiodo-L-tyrosine	0.22

ND = not detectable

Note: this table shows the cross-reactivity for the anti T3

### C. Precision

#### INTRA-ASSAY PRECISION

#### INTER-ASSAY PRECISION

Serum	N	<X> ± SD (nmol/l)	CV (%)	Serum	N	<X> ± SD (nmol/l)	CV (%)
A	10	1.06 ± 0.05	4.7	A	10	1.22 ± 0.05	3.7
B	10	5.49 ± 0.31	5.6	B	10	5.43 ± 0.16	3.0

SD: Standard Deviation; CV: Coefficient of variation

### D. Accuracy

#### DILUTION TEST

Sample	Dilution	Theoretical Concent. (nmol/l)	Measured Concent. (nmol/l)
A	1/1	-	12.29
	1/2	6.15	5.67
	1/4	3.07	2.98
	1/8	1.54	1.42
	1/16	0.77	0.72

Samples were diluted with zero calibrator.

#### RECOVERY TEST

Sample	added T3 (nmol/l)	Recovered T3 (nmol/l)	Recovered (%)
1	1	0.89	89%
	2	2.1	105%
	4	4.37	109%
	8	8.17	102%
	12	14.47	120%

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	1.04	1.07	1.05	1.10
C 2	5.15	5.74	6.13	5.76

## 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.

T3 concentrations for untreated euthyroid subjects ranged from 1.7 to 2.9 nmol/l (n=80).

## XVI. PRECAUTIONS AND WARNINGS

### Safety

For *in vitro* diagnostic use only.

This kit contains <sup>125</sup>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 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)	-	50	-
Samples, Controls	-	-	50
Tracer	200	200	200
Anti-T3	-	100	100
Incubation	1 hour at room temperature with continuous shaking		
Separation	-	Aspirate (or decant)	
Working Wash solution		2.0 ml	
Separation		Aspirate (or decant)	
Counting	Count tubes for 60 seconds		

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