FT3 Ria

KIRB1579

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

Distributed By:

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P
Minneapolis, Minnesota 55432, USA
Phone: (888) 523-1246
Fax.: (763) 780-2988
Email: info@ibl-america.com
Web: www.ibl-america.com

History

Summary of change :

Previous Version :	Current Version : 230915
	First version created

FT3 Ria

en

For the In Vitro Determination of Free Triiodothyronine in Human Serum and Plasma.

KIRB1579

Research Use Only

DIAsource ImmunoAssays SA - Rue du Bosquet 2, B-1348 Louvain-la-Neuve, Belgium - Tel: +32 10 84 99 11 - Fax : +32 10 84 99 90

PRINCIPLE OF THE ASSAY

The radioimmunoassay of free triiodothyronine (T3) is a competition assay based on the principle of labeled antibody. Samples and calibrators are incubated with ^{125}I-labeled monoclonal antibody specific for T3, as tracer, in tubes coated with an analog of T3 (ligand). There is competition between the free triiodothyronine of the sample and the ligand for the binding to the labeled antibody. After incubation, the content of tubes is aspirated and bound radioactivity is measured. A calibration curve is established and unknown values are determined by interpolation from the curve.

REAGENTS PROVIDED

All reagents of the kit are stable until the expiry date indicated on the kit labels, if stored at 2-8 °C. Expiry dates printed on vial labels apply to the long-term storage of components by the manufacturer only, prior to assembly of the kit. Do not take into account.

1 Kit for determination of free T3, 100 tubes

Coated tubes for the binding of the ligand : 2 x 50 tubes (ready-to-use)

Ab	125I

^{125}I-labeled monoclonal antibody: 1 x 45 mL vial (ready-to-use)
The vial contains 225 kBq, at the date of manufacture, of ^{125}I-labeled immunoglobulins in liquid form with bovine serum albumin, sodium azide (<0.1%; see § Precautions) and a dye.

CAL	N

Calibrators: 5 x 1 mL vials (ready-to-use)
The calibrator vials contain from 0 to approximately 44 pmol/L of free T3 in human serum (see § Precautions). The exact concentration is indicated on each vial label. Calibrators are verified to an internal reference calibrator.

CONTROL

Control serum: 1 x 1 mL vial (ready-to-use)
The vial contains T3 in human serum (see § Precautions). The expected values are in the concentration range indicated on the vial label.

MATERIAL REQUIRED BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:
- Precision micropipet (100 µL).
- Semi-automatic pipet (400 µL).
- Vortex type mixer.
- Horizontal or orbital shaker.
- Aspiration system.
- Gamma counter set for 125 iodine.

PRECAUTIONS

1. General remarks
- The vials with calibrators and controls should be opened as shortly as possible to avoid excessive evaporation.
- Do not mix the reagents from kits of different lots.
- A calibration curve must be included with each assay.
- The correct setting of the shaker is very important for the reproducibility of the assay.
- It is recommended to perform the assay in duplicate.
- Each tube must be used only once.

2. Basic rules of radiation safety
The purchase, possession, utilization, and transfer of radioactive material is subject to the regulations of the country of use.
Adherence to the basic rules of radiation safety should provide adequate protection:
- No eating, drinking, smoking or application of cosmetics should be carried out in the presence of radioactive materials.
- No pipeting of radioactive solutions by mouth.
- Avoid all contact with radioactive materials by using gloves and laboratory overalls.
- All manipulation of radioactive substances should be done in an appropriate place, distant from corridors and other busy places.
- Radioactive materials should be stored in the container provided in a designated area.
- A record of receipt and storage of all radioactive products should be kept up to date.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes.
- Each case of radioactive contamination or loss of radioactive material should be resolved according to established procedures.
- Radioactive waste should be handled according to the rules established in the country of use.
- This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.

3. Sodium azide
Some reagents contain sodium azide as a preservative. Sodium azide can react with lead, copper or brass to form explosive metal azides. Dispose of the reagents by flushing with large amounts of water through the plumbing system.

4. Material of human origin
The materials of human origin, contained in this kit, were found negative for the presence of antibodies to HIV 1 and HIV 2, antibodies to HCV, as well as of Hepatitis B surface antigen (HBsAg). However, they should be handled as if capable of transmitting disease. No known test method can offer total assurance that no virus is present. Handle this kit with all necessary precautions.
All serum and plasma samples should be handled as if capable of transmitting hepatitis or AIDS. Waste should be discarded according to the country rules.

SPECIMEN COLLECTION, PROCESSING AND STORAGE

-Collect blood in dry tubes or in tubes containing EDTA.
-Separate serum or plasma from cells by centrifugation.
-Serum and plasma samples may be stored at 2-8°C, if the assay is to be performed within 48 hours. For longer storage keep frozen (< -20°C, 3 months maximum) after aliquoting so as to avoid repeated freezing and thawing.
-Serum and EDTA plasma values for 20 samples (serum values ranging from 3.00 to 4.69 pM) were compared using the FT3 RIA kit. Results are as follows:
[EDTA-plasma] = 0.9844[serum]+0.2172
R = 0.9060

ASSAY PROCEDURE

ASSAY PROCEDURE

Bring all reagents to room temperature before pipetting.

Step 1 Additions *	Step 2 Incubation **	Step 3 Counting
To coated tubes add successively: - 100 µL of calibrator, control or sample and - 400 µL of tracer Mix	Incubate 120 minutes at 18-25°C with shaking (>280 rpm)	Aspirate carefully the content of tubes (except the 2 tubes «total cpm») Count bound cpm (B) and total cpm (T) for 1 min.

*** Add 400 µL of tracer to 2 additional tubes to obtain total cpm**.

** **An incubation time of 1 hour is sufficient if the test is performed automatically. 1-hour incubation values may not be the same in individual samples (see Appendix, § Correlation of 1-hour and 2-hour incubation procedure). The assay precision may be impacted as well.**

RESULTS

Results are obtained from the calibration curve by interpolation. The calibration curve serves for the determination of free T3 concentrations in samples measured at the same time as the calibrators.

1. Calibration curve

The results in the quality control department were calculated using spline curve fit with B/T or B/B0 on the logit vertical axis and analyte concentration of the calibrators on the log horizontal axis (pM).

Other data reduction methods may give slightly different results.

Total activity : 85959 cpm

Calibrators	FT3 (pmol/L)	cpm (n=3)	B/T (%)	B/B0 (%)
0	0	82116	95.5	100
1	2.10	68939	80.2	84.0
2	5.10	52225	60.8	63.6
3	10.4	34354	40.0	41.8
4	44.0	8890	10.3	10.8

(Example of calibration curve, do not use for calculation)

2. Samples

For each sample, locate the B/T (%) or B/B0 (%), on the vertical axis and read off the corresponding free T3 concentration on the horizontal axis.

To convert pmol/L into pg/mL, multiply results by **0.651**.

QUALITY CONTROL

Good laboratory practices imply that control samples be used regularly to ensure the quality of the results obtained. These samples must be processed exactly the same way as the assay samples, and it is recommended that their results be analyzed using appropriate statistical methods.

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address: products.support@diasource.be.

PERFORMANCE CHARACTERISTICS

(for more details, see the data sheet "APPENDIX")

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

1. Sensitivity

1.1 Analytical sensitivity: 0.5 pmol/L

1.2 Functional sensitivity: 1.0 pmol/L

2. Specificity

The antibody used in the immunoassay is highly specific for T3. Extremely low cross reactivities were obtained against several related molecules (L-T4, D-T4, T3r, etc).

3. Precision

3.1 Intra-assay

Samples were assayed in 15 times in the same series. The coefficients of variation were found below or equal to 6.4 % for serum samples.

3.2 Inter-assay

Samples were assayed in duplicate in 10 different series. Coefficients of variation were found below or equal to 5.5 % for serum samples.

4. Measurement range (from analytical sensitivity to highest calibrator):

0.5 to approximately 40 pmol/L.

LIMITATIONS OF THE PROCEDURE

The non-respect of the instructions in this package insert may affect results significantly.

Do not use hemolyzed, lipemic or icteric samples.

For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the specimen sample. Specimen which have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays.

Such interfering antibodies may cause erroneous results. Carefully evaluate the results of specimen suspected of having these antibodies.

Shortage of incubation time to 1 hour was tested on SR300 instrument. Performance characteristics of the assay are not guaranteed if different automate is used.

Revision date : 2023-09-15

Other translations of this Instructions for Use can be downloaded from our website: https://www.diasource-diagnostics.com/

APPENDIX

PERFORMANCE CHARACTERISTICS

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Specificity

The specificity of the monoclonal antibody was determined by a competition RIA, using labeled T3 and the following compounds:

Analog	Cross-reactivity (%)
L-3,3',5-triiodothyronine (T3)	100
L-3,3',5-triiodothyroacetic acid	100
L-3,3',5'-triiodothyronine (T3r)	0.03
L-thyroxine	0.15
D-thyroxine	0.07

Effective contribution to concentration of free thyroxine measured

Pooled normal human serum (assayed as 3.7 pM in free T3) was complemented with physiological or therapeutic concentrations of potentially interfering molecules. The free T3 concentration was measured with the Immunotech kit and the interfering contribution of each substance was calculated by subtraction of the free T3 concentration obtained in the absence of the interfering molecule.

Analog	Added	Free T3 equivalent (pM)
L-3,3',5-triiodothyronine (T3)	0.62 nM	< 0.1
L-3,3',5' -Triiodothyroacetic acid	0.089 nM	0.2
Mono-iodo-tyrosine	0.23 nM	< 0.1
Di-iodo-tyrosine	0.17 nM	0.2
Sodium Salicylate	1.25 mM	0.4

Precision

Intra-assay

EDTA-plasma	P1	P2	P3
Number of determinations	25	25	25
Mean value (pM)	3.09	9.99	19.07
C.V., %	3.94	6.50	4.11

Inter-assays

Serum	S1	S2	S3
Number of determinations	10	10	10
Mean value (pM)	2.71	4.45	11.3
C.V., %	5.53	5.50	3.62

EDTA-plasma	P1	P2	P3
Number of determinations	10	10	10
Mean value (pM)	1.77	16.73	30.63
C.V., %	12.18	5.24	4.19

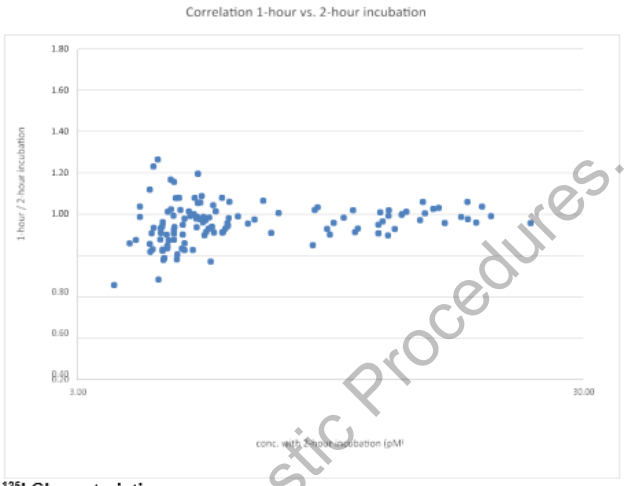
Correlation of 1-hour and 2-hour incubation procedure

Values of 122 samples (ranging from 3.54 to 23.6 pM) were determined using standard 2-hour and shortened 1-hour procedure on Stratec SR300. 1-hour incubation values may not be the same in individual samples as shown in the graph. The assay precision may be impacted as well.

Results were as follows:

[1-hour procedure] = 1.0062 x [2-hour procedure] – 0.2883

R = 0.9925



^{125}I Characteristics

$T_{1/2}$ (^{125}I) = 1443 h = 60.14 d

^{125}I	E (MeV)	%
γ	0.035	
X	0.027	114
	0.032	25