FT4 Ria

KIRB1363

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History

Summary of change :

Previous Version :	Current Version : 230915
	First version created

FT4 Ria

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

KIRB1363

Research Use Only

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PRINCIPLE OF THE ASSAY

The radioimmunoassay of free thyroxine (T4) is a competition assay based on the principle of labeled antibody. Samples and calibrators are incubated with ^{125}I-labeled monoclonal antibody specific for T4, as tracer, in the presence of a biotinylated analog of thyroxine (ligand) in avidin-coated tubes. There is competition between the free thyroxine of the sample and the ligand for the binding to the labeled antibody. The fraction of antibody complexed with the biotinylated ligand binds to avidin-coated tubes. 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 label, if stored at 2-8°C. Storage conditions for reagents after reconstitution or dilution are indicated in paragraph Assay Procedure.

Kit for determination of free T4, 100 tubes

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

Ab	125I

^{125}I-labeled monoclonal antibody: one 45 mL vial (ready-to-use)
The vial contains 310 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: five 0.5 mL vials (ready-to-use)
The calibrator vials contain from 0 to approximately 75 pmol/L of free T4 in human serum and sodium azide (<0.1%; see § Precautions). The exact concentration is indicated on each vial label. Calibrators are verified to an internal reference calibrator.

Ag	BIOT

Ligand: one 12 mL vial **(ready-to-use)**
The vial contains a ligand solution which includes also bovine proteins and sodium azide (<0.1% see § Precautions).

CONTROL

Control serum: one vial (lyophilised)
The vial contains T4 in human serum with sodium azide (<0.1%, see § Precautions). The expected values are in the concentration range indicated on the supplement.

Attention: All liquid reagents should be examined for the absence of precipitates; the antibody solution should be clear and blue-green, the calibrators may be opalescent and the ligand should be clear and colourless.

MATERIAL PROVIDED BUT NOT REQUESTED

In addition to standard laboratory equipment, the following items are required:
- Precision micropipette (25 µL).
- Semi-automatic pipets (100 µL and 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 and waste should be discarded according to the country rules.

SAMPLE COLLECTION, PROCESSING AND STORAGE

- Collect blood in dry tubes or in tubes containing EDTA, if possible after fasting.
- 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. Thawing of sample should be performed at room temperature. Serum and EDTA plasma values for 20 samples (serum values ranging from 14.18 to 22.43 pmol/L) were compared using the FT4 RIA kit. Results are as follows :
  {EDTA-plasma}=0.9872 (serum) + 0.2038
  R=0.955

ASSAY PROCEDURE

1. **Reconstitution of control serum**
   The contents of the vial must be brought to room temperature before reconstitution with the volume of distilled water indicated on the vial label. Wait for 10 min following reconstitution and mix gently to avoid foaming before dispensing. Store the reconstituted solution at 2-8°C for one week or aliquoted at <-18°C until the expiry date of the kit.

2. Assay procedure

ASSAY PROCEDURE
Bring all reagents to room temperature before pipeting.

Step 1 Additions *	Step 2 Incubation	Step 3 Counting
To coated tubes, add successively : - 25 µL of calibrators or samples and - 400 µL of tracer - 100 µL of ligand. Mix.	Incubate 60 min. at 18-25°C with shaking (>350 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.

RESULTS

Results are obtained from the standard curve by interpolation. The curve serves for the determination of free T4 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/BT or B/B0 on the logit vertical axis and analyte concentration of the calibrationon the log horizontal axis (pmol/l).

Other data reduction methods may give slightly different results.

Total activity: 100,549 cpm

Calibrators	free T4 (pmol/L)	cpm (n=3)	B/T (%)	B/B_0 (%)
0	0	65,929	65.7	100
1	2.6	51,766	51.5	78.5
2	12.1	26,994	26.8	40.9
3	29.4	7,522	7.51	11.4
4	83.0	1,415	1.41	2.15

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

2. Samples

For each sample, locate the B/T (%) or B/B_0 (%), on the vertical axis and read off the corresponding free T4 concentration in pmol/L on the horizontal axis.

To convert pmol/L to into ng/100 mL, multiply results by **0.0777.**

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: 0.4 pmol/L

1.2 Functional sensitivity: 2.34 pmol/L.

2. Specificity

The antibody used in the immunoassay is highly specific for T4. Extremely low cross reactivities were obtained against several related molecules (D-T4, T3, T3r, etc) or therapeutic drugs that may be present in specimen (Amiodarone etc).

3. Precision

3.1 Intra-assay

Samples were assayed in 25 times in the same series. The coefficients of variation were found below or equal to 10.29 % 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 7.58 % for serum samples.

4. Measurement range (from analytical sensitivity to highest calibrator):
0.4 to approximately – 75 pmol/L.

LIMITATIONS OF THE PROCEDURE

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

Plasma biotin concentrations of below 40 ng/mL do not interfere with the assay. In the case of specimen treated with high concentrations of biotin (5-10 mg/day), blood samples must be taken at least 8 hours after the last administration of biotin.

The kit has not been validated on neonatal specimens.

Do not use hemolyzed lipemic or icteric samples.

For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the 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.

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

APPENDIX

PERFORMANCE CHARACTERISTICS

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Specificity

Data on cross-reactivity with several related molecules for T4 were tested by RIA using 125I-labeled hormone and separating free from bound hormone by polyethylene glycol precipitation. The results are presented in the following table:

Analog	Cross-reactivity (%)
L-thyroxine	100
D-thyroxine	33
L-3,3',5-triiodothyronine (T3)	0.8
L-3,3',5'-triiodothyronine (T3r)	10.2

Precision

Intra-assay

Serum	S1	S2	S3
Number of determinations	25	25	25
Mean value, pM	5.17	15.31	29.46
C.V., %	10.29	3.06	3.11

EDTA Plasma	P1	P2	P3
Number of determinations	25	25	25
Mean value, pM	3.62	14.95	51.48
C.V., %	8.47	2.87	3.20

Inter-assay

Serum	S1	S2	S3
Number of determinations	10	10	10
Mean value, pM	5.62	27.95	42.23
C.V., %	7.58	3.94	2.54

EDTA Plasma	P1	P2	P3
Number of determinations	25	25	25
Mean value, pM	6.03	27.03	41.88
C.V., %	8.70	5.55	4.55

^{125}I Characteristics

T1/2 (125I) = 1443 h = 60.14 d

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