BECKMAN COULTER

CE 2797

FT4 RIA KIT

Instruction for use in local language is available at beckmancoulter.com/techdocs.

REVISION HISTORY

Previous version: PI-IM1363-3321-04	**Current version:** IFU-IM1363-3321-01
—	IVDR requirements incorporated
Chapter INTENDED USE removed	Chapter INTENDED PURPOSE added
—	Chapter APPENDIX: Interference data added

REF IM1363, IM3321

FOR PROFESSIONAL USE ONLY

INTENDED PURPOSE

FT4 RIA KIT is an in vitro diagnostic manual medical device intended to be used by healthcare professionals for the quantitative measurement of free thyroxine (FT4) in human serum and plasma. Measurement of free thyroxine is intended to be used as an aid in diagnosis of thyroid disorders in general population [1, 2, 3, 4].

PRINCIPLE

The radioimmunoassay of free thyroxine (FT4) is a competition assay. Samples and calibrators are incubated with ^{125}I-labeled monoclonal antibody, as a 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 contents of tubes are aspirated so as to remove unbound ^{125}I-labeled tracer. The bound radioactivity is then determined in a gamma counter. The FT4 concentrations in the samples are obtained by interpolation from the standard curve. The concentration of FT4 in the samples is indirectly proportional to the radioactivity.

WARNING AND PRECAUTIONS

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 standard curve must be established with each assay.
- It is recommended to perform the assay in duplicate.
- Each tube must be used only once.

Basic rules of radiation safety

The purchase, possession, utilization, and transfer of radioactive material are 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 pipetting 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.

Sodium azide

Some reagents contain sodium azide as a preservative. Sodium azide can react with lead, copper or brass to form explosive metal azides. Sodium azide disposal must be in accordance with appropriate local regulations.

Materials 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 patient specimens should be handled as potentially infectious and waste should be discarded according to the country rules.

GHS HAZARD CLASSIFICATION

Not classified as hazardous

SDS Safety Data Sheet is available at beckmancoulter.com/techdocs

SPECIMEN COLLECTION, PROCESSING, STORAGE AND DILUTION

- Serum or EDTA plasma are the recommended sample types.
- Allow serum samples to clot completely before 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 (at < -18°C, 1 year maximum), after aliquoting so as to avoid repeated freezing and thawing. Thawing of sample should be performed at room temperature.
- Dilution of samples with concentration greater than the highest calibrator is not recommended.

Serum and EDTA plasma values for 20 samples (serum values ranging from 14.18 to 22.43 pM) were compared using the IM1363 FT4 RIA KIT. Results are as follows:

[EDTA-plasma] = 0.9872[serum] + 0.2038, R = 0.977

MATERIALS PROVIDED

All reagents of the kit are stable until the expiry date indicated on the kit label, 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.

Storage conditions for reagents after reconstitution are indicated in paragraph Procedure.

Kit for determination of free T4, 100 tubes (REF. IM1363)

Tubes: 2 x 50 (ready-to-use)

^{125}I-Tracer: 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%) and a dye.

Calibrators: five 0.5 mL vials (ready-to-use)

The calibrator vials contain from 0 to approximately 75 pM of FT4 in human serum and sodium azide (<0.1%). The exact concentration is indicated on each vial label. The calibrators are traceable to an internal reference standard.

Ligand: one 12 mL vial (ready-to-use)

The vial contains a ligand solution which includes also bovine proteins and sodium azide (<0.1%).

Control sample: one vial (lyophilized)

The vial contains T4 in human serum with sodium azide (<0.1%). The concentration range is indicated on a supplement. The control sample is traceable to an internal reference standard.

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

Kit for determination of free T4, 400 tubes (REF. IM3321)

Tubes: 8 x 50 (ready-to-use)

^{125}I-Tracer: four 45 mL vials (ready-to-use)

Calibrators: five 0.5 mL vials (ready-to-use)

Ligand: four 12 mL vials (ready-to-use)

Control sample: one vial (lyophilized)

REAGENTS NOT PROVIDED

FT3 and FT4 Control sample: five vials (lyophilized)

Supplied upon request: REF. B48021

- The vials contain T3, T4 in human serum with sodium azide (<0.1%). The volume after reconstitution is 2 mL/vial.
- Control sample is intended as an optional additional one-level quality control to monitor the precision in determinations of Beckman Coulter RIA FT3 (IM1579, IM3320) and FT4 RIA KIT (IM1363, IM3321). This reagent can be used with any RIA FT3 or FT4 RIA KIT lot.

MATERIALS REQUIRED, BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:

- Precision micropipette (25 µL).
- Semi-automatic pipette (100 µL and 400 µL).
- Vortex type mixer.
- Horizontal or orbital shaker.
- Aspiration system.
- Gamma counter set for ^{125}I.

PROCEDURE

Preparation of reagents

Let all the reagents come to room temperature.

Reconstitution of control sample

The content of the vial is reconstituted with the volume of distilled water indicated on the 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 for a longer time, until the expiry date of the kit.

Assay procedure

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. Vortex gently 1-2 seconds.	Incubate 1 hour 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 minute.

*Add 400 µL of tracer to 2 additional tubes to obtain total cpm.
**An incubation time of 30 min at room temperature is sufficient if the test is performed automatically.

RESULTS

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

Standard curve

The results in the quality control department were calculated using *spline* curve fit with logit of *B/T or B/B_0* on the vertical axis and log of analyte concentration of the calibrators on the horizontal axis.

Other calculation methods may give slightly different results.

Total activity: 100,549 cpm				
Calibrators	**free T4 (pM)**	**cpm (n=3)**	**B/T (%)**	**B/B_0 (%)**
0	0	65,929	65.7	100.0
1	2.60	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 standard curve, do not use for calculation)

Samples

For each sample, locate ratio B/T or B/B_0 on the vertical axis and read off the corresponding analyte concentration on the horizontal axis.

To convert pmol/L (pM) to ng/100 mL, multiply results by 0.0777.

EXPECTED VALUES

We recommend each laboratory to establish its own reference values. The following values obtained from healthy subjects are indicative only.

11.5 – 23.0 pM

Remark: The following values were found on several studies on a total of 198 euthyroid patients.

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 in the same way as the assay samples, and it is recommended that their results be analyzed using appropriate statistical methods.

Failure to obtain the appropriate values for controls may indicate imprecise manipulations, improper sample handling or deterioration of reagents.

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: imunochem@beckman.com

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of EU Member State in which the user and/or patient is located.

PERFORMANCE CHARACTERISTICS

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

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

Sensitivity

Analytical sensitivity: 0.4 pM

Functional sensitivity: 2.34 pM

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 patient samples (Amiodarone).

Precision

Intra-assay

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

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.

Accuracy

It is generally accepted that the recovery, dilution and linearity tests may not provide quite satisfactory results when free hormones are determined.

Measurement range (from analytical sensitivity to the highest calibrator): 0.4 to approximately 75 pM.

LIMITATIONS

Failure to follow these instructions for use (IFU) may significantly affect results.

Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other appropriate information.

Do not use hemolyzed, lipemic or icteric samples. For more details, see Appendix, § Interference.

For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who 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 patients suspected of having these antibodies [5,6].

Shortage of incubation time to 30 minutes was tested on SR300 instrument. Performance characteristics of the assay are not guaranteed if different automate is used.

Plasma biotin concentrations of below 40 ng/mL do not interfere with the assay. In the case of patients 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.

APPENDIX

PERFORMANCE CHARACTERISTICS

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

Interference

Serum samples containing FT4 concentrations (low and high) were spiked with multiple concentrations of the substances listed below and assayed using FT4 RIA KIT. Values were calculated as described in CLSI EP07, 3rd ed. [7]. Interference was determined by testing controls (no interfering substance added) and matched test samples (with interfering substance added). No interference (defined as a shift in dose > 15 %) was found for addition of interferent up to concentration stated in the table below.

Interferent	Test concentration
Ascorbic acid	71.81 µg/mL
Conjugated bilirubin	451.7 µg/mL
Hemoglobin	10,464 µg/mL
Ibuprofen	392.9 µg/mL
Prednisone	149.6 ng/mL
Triglycerides	6.55 mg/mL
Unconjugated bilirubin	493.3 µg/mL

In spite of hemoglobin, bilirubin (conjugated, unconjugated) and triglyceride interference data in the table, we advise to avoid using hemolyzed, lipemic or icteric samples.

Specificity

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

Analogue	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	10	10	10
Mean value, pM	6.03	27.03	41.88
C.V., %	8.70	5.55	4.55

^{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

Symbols Key

REF — Product Reference / Référence du produit / Produktreferenz / Riferimento prodotto / Número de referencia del producto / Referência do produto / Produktreferens / Κωδικός αναφοράς προϊόντος / 产品参考 / Gaminio nuoroda / Termékszám / Dane referencyjne produktu / Reference k produktu / Referenčné označenie výrobku / 제품 참조 자료 / Ürün Referansı / Ссылка на продукт / Референца за производ / 產品參考

IVD — In Vitro Diagnostic / Diagnostic in vitro / In-vitro-Diagnostikum / Diagnostica in vitro / Para diagnóstico in vitro / Diagnóstico in vitro / InVitro-diagnostik / Για διάγνωση in vitro / 体外诊断 / In vitro diagnostika / In vitro diagnosztikai felhasználásra / Diagnostyka in vitro / Diagnostika in vitro / 체외 진단 / İn Vitro Diagnostik / Диагностика in vitro / За ин витро диагностика / 體外診斷

CONTENTS — Contents / Contenu / Inhalt / Contenuto / Contenido / Conteúdo / Περιεχόμενο / 组成 / Rinkinio sudėtis / Tartalom / Zawartość / Obsah / Obsah / 내용물 / İçindekiler / Содержание / Съдържание / 目錄

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Ag 125I / Ab 125I — Tracer / Traceur / Tracer / Marcato / Trazador / Marcador / Tracer / Ανιχνευτής / 追踪剂 / Atsekamoji medžiaga / Nyomjelző / Znacznik / Radioindikátor / Indikátor (tracer) / 트레이서 / Tracer´lar / метка / Индикатор / 追蹤劑

CAL / CAL 0 — Calibrator / Calibrateur / Kalibrator / Calibratore / Calibrador / Calibrador / Kalibrator / Βαθμονομητής / 校准品 / Kalibravimo medžiaga / Kalibrátor / Kalibrator / kalibrátor / Kalibrátor / 보정 물질 / Kalibratör / Калибратор / Калибратор / 校正液

CTRL — Control / Contrôle / Kontrolle / Controllo / Control / Controlo / Kontrolle / Μάρτυρας / 质控品 / Kontrolinė / Kontroll / Kontrola / Kontrola / Kontrola / 정도관리 / Kontrol / Контроль / Контролна / 質控品

TUBE — Tubes / tubes / Röhrchen / provette / tubos / Tubos de amostra / Provrör / σωληνάρια / 试管 / Mėgintuvėliai / Csövek / Probówki / Zkumavky / Skúmavky / 튜브 / Tüpler / пробирки / Епруветки / 試管

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LIGND — Ligand / Ligand / Ligand / Ligando / Ligando / Ligand / Συνδέτης / 配体 / Ligandas / Ligandum / Ligand / Ligand / 리간드 / Ligand / Лиганд / Лиганд / 配體

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