Application Sheet

Laboratory Name
Test Name: FT4 II

REF	Σ	SYSTEM
06437281 160	200	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

For USA: Elecsys FT4 II Assay

System information

For **cobas e** 411 analyzer: test number 1250
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 201

Intended use

Assay for the in vitro quantitative determination of free thyroxine in human serum and plasma. Measurements obtained by this device are used in the diagnosis and treatment of thyroid disease. The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Thyroxine (T4) is the main thyroid hormone secreted into the bloodstream by the thyroid gland. Together with triiodothyronine (T3) it plays a vital role in regulating the body's metabolic rate, influences the cardiovascular system, growth and bone metabolism, and is important for normal development of gonadal functions and nervous system.[1]
T4 circulates in the bloodstream as an equilibrium mixture of free and serum bound hormone. Free T4 (fT4) is the unbound and biologically active form, which represents only 0.03 % of the total T4. The remaining T4 is inactive and bound to serum proteins such as thyroxine binding globulin (75 %), pre-albumin (15 %), and albumin (10 %).[2,3,4,5]
The determination of free T4 has the advantage of being independent of changes in the concentrations and binding properties of the binding proteins; additional determination of a binding parameter (T-uptake, TBG) is therefore unnecessary. Therefore free T4 is a useful tool in clinical routine diagnostics for the assessment of the thyroid status. It should be measured together with TSH if thyroid disorders are suspected and is also suitable for monitoring thyrosuppressive therapy.[1,6,7]
A variety of methods are available for estimating the free thyroid hormone levels. The direct measurement of fT4 and fT3 via equilibrium dialysis or ultrafiltration is mainly used as a reference method for standardizing the immunological procedures generally used for routine diagnostic purposes.[6,7]
In the Elecsys FT4 II assay a specific anti-T4 antibody labeled with a sulfonyl-ruthenium complex[a)] is used to determine the free thyroxine.
a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Test principle

Competition principle. Total duration of assay: 18 minutes.
- 1st incubation: 15 µL of sample and a T4-specific antibody labeled with a sulfonyl-ruthenium complex.
- 2nd incubation: After addition of biotinylated T4 and streptavidin-coated microparticles, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission

which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Reagents - working solutions

The reagent rackpack is labeled as FT4 II.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-T4-Ab~Ru(bpy)$_3^{2+}$ (gray cap), 1 bottle, 18 mL:
Polyclonal anti-T4-antibody (sheep) labeled with ruthenium complex 75 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 T4~biotin (black cap), 1 bottle, 18 mL:
Biotinylated T4 2.5 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.
Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.
Do not freeze.
Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	84 days (12 weeks)
on the analyzers	28 days (4 weeks) onboard or 56 days (8 weeks) when stored alternately in the refrigerator and on the analyzer, with the total time onboard the analyzer not exceeding 120 hours

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.
Undiluted serum collected using standard sampling tubes or tubes containing separating gel.
Undiluted Li-heparin, K_2-EDTA and K_3-EDTA plasma.
Criterion: Recovery with a total deviation ≤ ± 0.05 ng/dL (0.6 pmol/L) of initial value at concentrations < 0.3 ng/dL (3.9 pmol/L); recovery within ± 10 % of initial value at concentrations ≥ 0.3 ng/dL (3.9 pmol/L) and slope 0.9-1.1 + intercept within ≤ ± 0.05 ng/dL + coefficient of correlation ≥ 0.95.
Stable for 7 days at 2-8 °C, 30 days at -20 °C.[6] Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay.
Do not use heat-inactivated samples.
Do not use samples and controls stabilized with azide.
Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.
The claims, including those pertaining to sample stability made in the labeling of the cleared/approved reagents of Roche Diagnostics are part of the clearance of the overall IVD test system (assay). Sample stability was tested only for the temperatures/time frame as claimed by the manufacturer under the conditions claimed in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.
Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- REF 06437290190, FT4 II CalSet, 4 x 1 mL or
  REF 06437290160, FT4 II CalSet, 4 x 1 mL
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500160, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

In addition, other suitable control material can be used.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.
Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).
MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:
PreClean M solution is necessary.
Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the Elecsys FT4 method. The Elecsys FT4 assay is traceable to the Enzymun-Test which was standardized using equilibrium dialysis.[5,8]
Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.
Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).
Calibration interval may be extended based on acceptable verification of calibration by the laboratory.
Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

At least once daily run solutions at two levels of a quality control material with known concentrations.

Refer to Brown Clinic Quality Control Requirements, Rules and Reviews Policy

Refer to Brown Clinic Quatliy Control Specialty and Subspecialty Policy

Calculation

The analyzer automatically calculates the analyte concentration of each sample either in pmol/L, ng/dL or ng/L.

Conversion factors:
pmol/L x 0.077688 = ng/dL
ng/dL x 12.872 = pmol/L
pmol/L x 0.77688 = ng/L

Limitations - interference

The assay is unaffected by icterus (bilirubin < 701 µmol/L or < 41 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 2000 mg/dL), biotin (< 81.8 nmol/L or < 20 ng/mL), albumin < 6.3 g/dL, IgG < 7 g/dL, IgA < 1.6 g/dL and IgM < 1 g/dL.
Criterion: Recovery within ± 10 % of initial value.
Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
No interference was observed from rheumatoid factors up to a concentration of 1200 IU/mL and samples from dialysis patients.
Any influence that might affect the binding behavior of the binding proteins can alter the result of the fT4 tests (e.g. drugs, NTIs (Non-Thyroid-Illness) or patients suffering from FDH (Familial Dysalbuminemic Hyperthyroxinemia) or increased TBG in pregnancy).[9,10,11]
The test cannot be used in patients receiving treatment with lipid-lowering agents containing D-T4. If the thyroid function is to be checked in such patients, the therapy should first be discontinued for 4-6 weeks to allow the physiological state to become re-established.[12]
Autoantibodies to thyroid hormones can interfere with the assay.[7]
In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.
The following special thyroid drugs were tested with concentrations shown in the table below. No interference with the assay was found.
Criterion: Recovery within ± 10 % of initial value.

Drug	**Concentration (µg/mL)**
Iodide	0.200
Carbimazole	6

Drug	Concentration (µg/mL)
Thiamazole	80
Propylthiouracil	300
Perchlorate	2000
Propranolol	240
Amiodarone	200
Prednisolone	100
Hydrocortisone	200
Fluocortolone	100
Octreotide	0.300

In in vitro studies the drugs Furosemide and Levothyroxine caused elevated fT4 findings at the daily therapeutic dosage level.
In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range
0.101-7.77 ng/dL (1.3-100 pmol/L) (defined by the Limit of Quantitation (functional sensitivity) and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 0.101 ng/dL (1.3 pmol/L). Values above the measuring range are reported as > 7.77 ng/dL (100 pmol/L).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation
Limit of Blank = 0.03 ng/dL (0.4 pmol/L)
Limit of Detection = 0.05 ng/dL (0.6 pmol/L)
Limit of Quantitation (functional sensitivity) = 0.101 ng/dL (1.3 pmol/L) with an intermediate precision of ≤ 20 %
The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.
The Limit of Blank is the 95^{th} percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.
The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).
The Limit of Quantitation (functional sensitivity) is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples for fT4 determinations cannot be diluted, as T4 in the blood is present in free and protein-bound forms which are in equilibrium. A change in the concentration of the binding proteins alters this equilibrium.

Expected values

Euthyroid: 0.93-1.7 ng/dL (12-22 pmol/L)

These values correspond to the 2.5th and 97.5th percentile of results from a total of 801 healthy test subjects studied.
Status: MCE Reference Range Thyroid, Status 1st quarter 1998.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

For Known Interfering Substances section refer to package insert.
For Known Non-Interfering Substance refer to package insert.
For Additional Technical Information refer to package insert.

References

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Alternative method

Refer to Brown Clinic Back-up Testing Policy

Source document

Reagent Name: FT4 II
Method Sheet Version: V3.0 English

Effective date

Effective date for this procedure:

Author

Source documentation compiled by Roche Diagnostics

Revised by: Heather J Hall, MBA, MT(ASCP), CG(ASCP)[cm] Date: 4/9/2018

Approved by: Aaron Shives MD (Signature on file Date: 4/11/2018

REVIEW – REVISION SUMMARY DOCUMENTATION

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