

REF		SYSTEM
06437281 190	200	Elecsys 2010 MODULAR ANALYTICS E170 cobas e 411 cobas e 601 cobas e 602

English**Intended use**

Immunoassay for the in vitro quantitative determination of free thyroxine in human serum and plasma.

The electrochemiluminescence immunoassay "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 these 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 thyrostatic 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 ruthenium complex^{a)} is used to determine the free thyroxine.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 15 µL of sample and a T4-specific antibody labeled with a 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.

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)₃²⁺ (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.

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 alternatively in the refrigerator and on the analyzer, with the total time onboard on 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, K₂-EDTA and K₃-EDTA plasma.

Criterion: Recovery with a total deviation ≤ ± 0.6 pmol/L of initial value at concentrations < 3 pmol/L; recovery within ± 10 % of initial value at concentrations ≥ 3 pmol/L and slope 0.9-1.1 + intercept within ≤ ± 2x Limit of Blank (LoB) + 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.

Free thyroxine

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
- [REF] 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- [REF] 11662988122, ProCell, 6 x 380 mL system buffer
- [REF] 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- [REF] 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- [REF] 11933159001, Adapter for SysClean
- [REF] 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- [REF] 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

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 Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- [REF] 03023150001, WasteLiner, waste bags
- [REF] 03027651001, SysClean Adapter M

Accessories for all analyzers:

- [REF] 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

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.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approx. 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). 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

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

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), 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)).^{9,10}

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.¹¹

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)
Iodid	0.200
Carbimazol	6
Thiamazol	80
Propylthiouracil	300
Perchlorat	2000
Propranolol	240
Amiodaron	200
Prednisolon	100
Hydrocortison	200
Fluocortolon	100
Octreotid	0.300

In in vitro studies the drugs Furosemide and Levothyroxine caused elevated FT4 findings at the daily therapeutic dosage level.

Free thyroxine

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**

0.3-100 pmol/L (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.3 pmol/L. Values above the measuring range are reported as > 100 pmol/L.

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Blank = 0.3 pmol/L

Limit of Detection = 0.5 pmol/L

Limit of Quantitation = 3 pmol/L with a total allowable error of ≤ 30 %

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from $n \geq 60$ 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 is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error of ≤ 30 %.

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

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.

For detailed information about reference intervals in children, adolescents and pregnant women, refer to the brochure "Reference Intervals for Children and Adults", English: [REF] 04640292, German: [REF] 04625889.

This booklet also contains results of a detailed study about influencing factors on thyroid parameters in a well characterized reference group of adults.

Different inclusion and exclusion criteria were applied (e.g. sonographic results (thyroid volume and density) as well as criteria according to the guidelines of the National Academy of Clinical Biochemistry - NACB).

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

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days ($n = 84$). The following results were obtained:

Elecsys 2010 and cobas e 411 analyzers					
Sample	Mean pmol/L	Repeatability		Intermediate precision	
		SD pmol/L	CV %	SD pmol/L	CV %
HS ^{b)} 1	1.78	0.072	4.0	0.136	7.6
HS 2	13.3	0.169	1.3	0.301	2.3
HS 3	24.5	0.307	1.3	0.518	2.1
HS 4	63.5	1.06	1.7	2.10	3.3
HS 5	91.2	1.63	1.8	4.11	4.5
PC U ^{c)} 1	15.7	0.139	0.9	0.279	1.8
PC U2	40.0	0.417	1.0	1.15	2.9

b) HS = human serum

c) PC = PreciControl Universal

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
Sample	Mean pmol/L	Repeatability		Intermediate precision	
		SD pmol/L	CV %	SD pmol/L	CV %
HS 1	1.58	0.080	5.0	0.100	6.3
HS 2	13.1	0.213	1.6	0.217	2.1
HS 3	24.1	0.381	1.6	0.516	2.1
HS 4	61.9	1.26	2.0	2.03	3.3
HS 5	87.8	2.33	2.7	3.40	3.9
PC U1	15.6	0.278	1.8	0.293	1.9
PC U2	39.3	0.646	1.6	0.913	2.3

Method comparison

A comparison of the Elecsys FT4 II assay (y) with the Elecsys FT4 assay (x) using clinical samples gave the following correlations:

Number of samples measured: 170

Passing/Bablok¹² Linear regression

$y = 0.978x - 0.142$

$y = 1.02x - 0.963$

$\tau = 0.930$

$r = 0.996$

The sample concentrations were between approx. 1.66 and 92.3 pmol/L.

Analytical specificity

The following cross-reactivities were found, tested with fT4 concentrations of approx. 12.5 pmol/L (0.974 ng/dL) and 34.2 pmol/L (2.66 ng/dL):

Cross-reactant	Concentration tested ng/dL	Cross-reactivity %
L-T3	50000	0.005
D-T3	50000	0.001
rT3	190000	0.003
3-iodo-L-tyrosine	10000000	0.000
3,5-diiodo-L-tyrosine	10000000	0.000
3,3',5-triiodothyroacetic acid	100000	0.0002
3,3',5,5'-tetraiodothyroacetic acid	100000	0.001

References

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Free thyroxine

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume after reconstitution or mixing

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