Elecsys FT4 IV

cobas®

REF		Σ	SYSTEM
			cobas e 411
09043276190	09043276500	200	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 2210
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 302

Intended use

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

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Free thyroxine (fT4) measurements, performed with this assay, in human serum and plasma are used as an aid in diagnosis of thyroid disorders.

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 protein-bound hormone. 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 (TBG), thyroxine-binding prealbumin or transthyretin (TTR), and albumin.[2]

The determination of fT4 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. FT4 is a useful tool in clinical routine diagnostics for the assessment of the thyroid function. It should be measured together with TSH if thyroid disorders are suspected and is also suitable for monitoring treatment of secondary hypothyroidism (due to pituitary or hypothalamic disease) and of hyperthyroidism.[1,2,3,4,5,6]

A variety of methods are available for estimating the free thyroid hormone levels.[2,3] In the Elecsys FT4 IV assay, a T4-specific antibody labeled with a 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 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 4.

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:
Monoclonal anti-T4 antibody (rabbit) 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 for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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

Elecsys FT4 IV

cobas®

Stability:	
on the analyzers	28 days onboard or 56 days 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.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 0.6 pmol/L + coefficient of correlation ≥ 0.95.

Stable for 5 days at 20-25 °C, 7 days at 2-8 °C, 30 days at -20 °C (± 5 °C). 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.

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 09043292190, CalSet FT4 IV, 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **cobas e** 411 analyzer:

- 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, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **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 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/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.

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 was standardized against the Elecsys FT4 III method. The Elecsys FT4 III assay is traceable to the Elecsys FT4 II assay which in turn is traceable to the Enzymun Test FT4 which has been standardized using equilibrium dialysis.[7,8]

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

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.

If necessary, repeat the measurement of the samples concerned.

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 effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 701 µmol/L or ≤ 41 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Compound	Concentration tested
IgG	≤ 7 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1 g/dL

Criterion: Recovery of ≤ ± 0.6 pmol/L of initial value ≤ 6 pmol/L and ± 10 % of initial value > 6 pmol/L.

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.[12]

Pharmaceutical substances
In vitro tests were performed on 14 commonly used pharmaceuticals. No interference with the assay was found. For the common pharmaceuticals phenylbutazone, itraconazol and ibuprofen no interference was observed for concentrations ≤ 80 µg/mL, ≤ 15 µg/mL and ≤ 109 µg/mL, respectively.

In addition, the following special thyroid drugs were tested. For the stated concentrations, no interference with the assay was found. The drugs furosemide, carbamazepine, phenytoin and levothyroxine sodium (L-T4, synthetic levothyroxine[13]) caused elevated fT4 recovery at the daily therapeutic concentration.

Special thyroid drugs

Drug	Concentration tested µg/mL
Iodide	0.2
Carbimazole	18
Thiamazole	80
Propylthiouracil	300
Perchlorate	600
Propranolol	120
Amiodarone	200
Prednisolone	100
Hydrocortisone	200
Octreotide	0.3
Furosemide	3.5
Liothyronine	0.02
Potassium iodide (SSKI)	150
Lithium	540
Phenytoin	13.5
Carbamazepine	9

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

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

0.5-100 pmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.5 pmol/L. Values above the measuring range are reported as > 100 pmol/L.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.3 pmol/L

Limit of Detection = 0.5 pmol/L

Limit of Quantitation = 1.3 pmol/L

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 95th 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 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

11.9-21.6 pmol/L (0.92-1.68 ng/dL)

These values correspond to the 2.5th and 97.5th percentiles of results obtained from a total of 150 apparently healthy subjects examined (Roche study No. RD005427, 2021).
Corresponding lower and upper confidence limits (95 % CI[b)]) of the 2.5th and 97.5th percentiles:

2.5th percentile	95 % CI of the 2.5th percentile	97.5th percentile	95 % CI of the 97.5th percentile	Unit
11.9	10.4-12.3	21.6	19.4-25.8	pmol/L
0.92	0.81-0.96	1.68	1.51-2.00	ng/dL

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.

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.

b) CI = confidence interval

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 (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

Elecsys FT4 IV

cobas®

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	1.59	0.040	2.5	0.089	5.6
Human serum 2	12.6	0.133	1.1	0.248	2.0
Human serum 3	23.4	0.222	1.0	0.402	1.7
Human serum 4	54.8	0.705	1.3	1.31	2.4
Human serum 5	97.6	1.53	1.6	2.75	2.8
PC[c)] Universal 1	15.1	0.131	0.9	0.265	1.7
PC Universal 2	39.8	0.482	1.2	0.824	2.1

c) PC = PreciControl

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	1.86	0.077	4.1	0.100	5.4
Human serum 2	12.5	0.171	1.4	0.223	1.8
Human serum 3	22.9	0.241	1.1	0.361	1.6
Human serum 4	53.6	0.489	0.9	1.00	1.9
Human serum 5	95.7	1.79	1.9	2.84	3.0
PC Universal 1	15.0	0.205	1.4	0.244	1.6
PC Universal 2	39.0	0.437	1.1	0.742	1.9

Method comparison

A comparison of the Elecsys FT4 IV assay, REF 09043276190 (**cobas e** 601 analyzer; y), with the Elecsys FT4 III assay, REF 07976836190 (**cobas e** 601 analyzer; x) gave the following correlations (pmol/L):

Number of samples measured: 121

Passing/Bablok[14]	Linear regression
y = 1.013x - 0.062	y = 0.972x + 0.785
τ = 0.961	r = 0.998

The sample concentrations were between 1.40 and 97.0 pmol/L.

Analytical specificity

The following cross-reactivities were found, tested with fT4 concentrations of approximately 19.4 pmol/L and 64.5 pmol/L:

Cross-reactant	Concentration tested ng/dL	Cross-reactivity %
L-T3	50000	0.005
D-T3	50000	0.003
rT3	190000	0.002
3-iodo-L-tyrosine	10000000	0.000
3,5-diiodo-L-tyrosine	10000000	0.000
3,3',5-triiodothyroacetic acid	100000	0.000
3,3',5,5'-tetraiodothyroacetic acid	100000	0.003

References

1 Kronenberg HM, Melmed S, Polonsky KS, et al. Williams Textbook of Endocrinology. Saunders Elsevier, Philadelphia, 12th edition, 2011, chapter 10, p. 301-311.

2 Ellervik C, Halsall DJ, Nygaard B. Thyroid Disorders. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 57, p. 806-845.e13.

3 Brent GA. Thyroid Function Testing. Springer, Berlin, 1st edition, 2010, chapter 5, p. 86-88.

4 Bartalena L, Bogazzi F, Chiovato L, et al. 2018 European Thyroid Association (ETA) Guidelines for the Management of Amiodarone-Associated Thyroid Dysfunction. Eur Thyroid J 2018 Mar;7(2):55-66. doi: 10.1159/000486957.

5 Persani L, Brabant G, Dattani M, et al. 2018 European Thyroid Association (ETA) Guidelines on the Diagnosis and Management of Central Hypothyroidism. Eur Thyroid J 2018 Oct;7(5):225-237. doi: 10.1159/000491388.

6 Kahaly GJ, Bartalena L, Hegedüs L, et al. 2018 European Thyroid Association Guideline for the Management of Graves' Hyperthyroidism. Eur Thyroid J 2018 Aug;7(4):167-186. doi: 10.1159/000490384.

7 Ekins RP. Measurement of free hormones in blood. Endocr Rev 1990;11(1):5-46.

8 Ekins RP, Ellis SM. The radioimmunoassay of free thyroid hormones in serum. In Robbins J, Braverman LE (eds). Thyroid research, Proceedings of the Seventh International Thyroid Conference, Boston. Amsterdam, Excerpta Medica 1975:597.

9 Wada N, Chiba H, Shimizu C, et al. A novel missense mutation in codon 218 of the albumin gene in a distinct phenotype of familial dysalbuminemic hyperthyroxinemia in a Japanese kindred. J Clin Endocrinol Metab 1997;82(10):3246-3250.

10 Arevalo G. Prevalence of familial dysalbuminemic hyperthyroxinemia in serum samples received for thyroid testing. Clin Chem 1991;37(8):1430-1431.

11 Bantle JP, Hunninghake DB, Frantz ID, et al. Comparison of effectiveness of thyrotropin-suppressive doses of D- and L-thyroxine in treatment of hypercholesterolemia. Am J Med 1984;77(3):475-481.

12 Brent GA. Thyroid Function Testing. Springer, Berlin, 1st edition, 2010, chapter 5, p. 86-101.

13 Hennessey JV. The emergence of levothyroxine as a treatment for hypothyroidism. Endocrine 2017;55(1):6-18.

14 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets 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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

09043276500V2.0

Elecsys FT4 IV

Additions, deletions or changes are indicated by a change bar in the margin.

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606