

Elecsys FT4 IV

REF			SYSTEM
09043284190	09043284501	300	cobas e 402 cobas e 801

English

For use in the USA only

System information

Short name	ACN (application code number)
FT4 4	10195

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 electrochemiluminescence immunoassay "ECLIA" is intended for use on **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 proteins 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, TBG (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. Thus free T4 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 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 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)₃²⁺)

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 9 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 II 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 **cobas** link.

Reagents - working solutions

The **cobas e** pack is labeled as FT4 4.

- M Streptavidin-coated microparticles, 1 bottle, 13.2 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

- R1 Anti-T4-Ab-Ru(bpy)₃²⁺, 1 bottle, 19.7 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, 1 bottle, 19.7 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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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: 1-800-428-2336

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 available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Elecsys FT4 IV

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

The following specimens were tested and found acceptable: serum, Li-heparin plasma, K₂-EDTA and K₃-EDTA plasma.

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.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated 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.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 09043292160, CalSet FT4 IV, 4 x 1.0 mL
- REF 11731416160, PreciControl Universal, for 4 x 3.0 mL
- General laboratory equipment
- cobas e** analyzer

Additional materials for the **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500160, 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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: Elecsys FT4 IV is traceable to Elecsys FT4 II. The Elecsys FT4 II assay is traceable to the Enzymun Test FT4 which has been standardized using equilibrium dialysis.^{5,8}

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 **cobas e** pack 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 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack 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 **cobas e** pack, 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.

$$\begin{aligned} \text{Conversion factors:} \quad & \text{pmol/L} \times 0.077688 = \text{ng/dL} \\ & \text{ng/dL} \times 12.872 = \text{pmol/L} \\ & \text{pmol/L} \times 0.77688 = \text{ng/L} \end{aligned}$$

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
IgG	≤ 7 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1 g/dL
Albumin	≤ 6.3 g/dL

Criterion: Recovery of ≤ ± 0.05 ng/dL (0.6 pmol/L) of initial value ≤ 0.47 ng/dL (6 pmol/L) and ± 10 % of initial value > 0.47 ng/dL (6 pmol/L).

Specimens that contain biotin at a concentration of 1200 ng/mL demonstrate less than or equal to 10 % bias. Pharmacokinetic studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day⁹ and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.¹⁰

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).^{11,12,13}

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.¹⁴

Autoantibodies to thyroid hormones can interfere with the assay.⁷

Pharmaceutical substances

In vitro tests were performed on 17 commonly used pharmaceuticals. No significant interference was found with the highest concentration tested listed in the table below.

Common therapeutic drugs	Concentration tested µg/mL
Acetylcysteine	150
Ampicillin	75
Ascorbic Acid	52.5
Cyclosporine	1.8
Cefoxitin	750
Heparin	3300 IU/L
Itraconazole	15
Levodopa	7.5
Methyldopa	22.5
Metronidazole	123
Phenylbutazone	80
Doxycycline	18
Acetylsalicylic Acid	30
Rifampicin	48
Acetaminophen	156
Ibuprofen	109
Theophylline	60

Special thyroid drugs

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 levothyroxine¹⁵) caused elevated FT4 recovery at the daily therapeutic concentration.

Drug	Concentration tested µg/mL
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

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 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 = 0.02 ng/dL (0.3 pmol/L)

Limit of Detection = 0.04 ng/dL (0.5 pmol/L)

Limit of Quantitation = 0.101 ng/dL (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 $n \geq 60$ 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 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

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

A reference range study was conducted with samples from apparently healthy donors from the United States. 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:

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

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:

cobas e 402 and cobas e 801 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean ng/dL (pmol/L)	SD ng/dL (pmol/L)	CV %	SD ng/dL (pmol/L)	CV %
Human serum 1	0.135 (1.74)	0.006 (0.080)	4.6	0.010 (0.130)	7.5
Human serum 2	0.504 (6.49)	0.008 (0.106)	1.6	0.016 (0.206)	3.2
Human serum 3	0.948 (12.2)	0.016 (0.205)	1.7	0.025 (0.321)	2.6
Human serum 4	1.76 (22.6)	0.019 (0.248)	1.1	0.042 (0.546)	2.4
Human serum 5	3.42 (44.0)	0.040 (0.519)	1.2	0.077 (0.994)	2.3
Human serum 6	6.95 (89.4)	0.245 (3.16)	3.5	0.293 (3.77)	4.2
PC ^{c)} Universal 1	1.18 (15.2)	0.015 (0.199)	1.3	0.024 (0.304)	2.0
PC Universal 2	3.12 (40.2)	0.043 (0.548)	1.4	0.062 (0.799)	2.0

c) PC = PreciControl

Method comparison

a) A comparison of the Elecsys FT4 IV assay, [REF] 09043276160 (cobas e 411 analyzer; y), with the Elecsys FT4 II assay, [REF] 06437281160 (cobas e 411 analyzer; x), gave the following correlations (ng/dL):

Number of samples measured: 121

Passing/Bablok¹⁶ Linear regression

$$y = 1.03x - 0.025$$

$$y = 1.04x - 0.034$$

$$r = 0.967$$

$$r = 0.999$$

The sample concentrations were between 0.13 and 7.68 ng/dL (1.65 and 98.8 pmol/L).

b) A comparison of the Elecsys FT4 IV assay, [REF] 09043284190 (cobas e 801 analyzer; y), with the Elecsys FT4 IV assay, [REF] 09043276160 (cobas e 601 analyzer; x), gave the following correlations (ng/dL):

Number of samples measured: 121

Passing/Bablok¹⁶ Linear regression

$$y = 0.989x - 0.016$$

$$y = 1.005x - 0.034$$

$$r = 0.964$$

$$r = 0.999$$

The sample concentrations were between 0.10 and 7.63 ng/dL (1.30 and 98.2 pmol/L).

Analytical specificity

The following cross-reactivities were found, tested with FT4 concentrations of approximately 1.51 ng/dL (19.4 pmol/L) and 5.01 ng/dL (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

Cross-reactant	Concentration tested ng/dL	Cross-reactivity %
3,3',5,5'-tetraiodothyroacetic acid	100000	0.003

References

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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).

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

Elecsys FT4 IV



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

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