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FT4

Free thyroxine

cobas®

REF	Σ	SYSTEM
		Elecsys 2010 MODULAR ANALYTICS E170
11731297 122	200	**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 **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

The thyroid hormone thyroxine (T4) is physiologically part of the regulating circuit of the thyroid gland and has an effect on general metabolism. The major fraction of the total thyroxine is bound to transport proteins (TBG, prealbumin, and albumin). The free thyroxine (fT4) is the physiologically active thyroxine component.

The determination of free thyroxine is an important element in clinical routine diagnostics. Free T4 is measured together with TSH when thyroid function disorders are suspected. The determination of fT4 is also suitable for monitoring thyrosuppressive therapy.[1,2]

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.

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.[3,4]

In the Elecsys FT4 assay the determination of free thyroxine is made with the aid of a specific anti-T4 antibody labeled with a ruthenium complex[a)]. The quantity of antibody used is so small (equivalent to approx. 1-2 % of the total T4 content of a normal serum sample) that the equilibrium between bound and unbound T4 remains virtually unaffected.

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.

Reagents - working solutions

The reagent rackpack is labeled as FT4.

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 50 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	12 weeks
on the analyzers	3 weeks or 42 days when stored alternatively in the refrigerator and on the analyzer, with the total time onboard on the analyzer not exceeding 80 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.

Li-, Na-, NH_4^+-heparin, K_3-EDTA, sodium citrate and sodium fluoride/potassium oxalate plasma (undiluted).

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 7 days at 2-8 °C, 30 days at -20 °C.[5] Freeze only once.

Stability of serum obtained with tubes containing separating gel:

48 hours at 2-8 °C (note the data provided by the tube manufacturer).

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.

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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 11731661122, FT4 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 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.

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 Enzymun-Test FT4 method. This in turn was standardized using equilibrium dialysis.[3,4]

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 < 1.2 mmol/L or < 2 g/dL), lipemia (Intralipid < 2000 mg/dL), biotin (< 409 nmol/L or < 100 ng/mL), IgG < 7 g/dL and IgM < 2 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 339 IU/mL and samples from dialysis patients.

Of 26 commonly used pharmaceuticals tested in vitro, only furosemide caused elevated fT4 findings at the daily therapeutic dosage level.

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.[6]

Autoantibodies to thyroid hormones can interfere with the assay.

Binding protein anomalies seen with FDH (familial dysalbuminemic hyperthyroxinemia), for example, may cause values which, while characteristic of the condition, deviate from the expected results.[7]

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

0.300-100 pmol/L or 0.023-7.77 ng/dL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.300 pmol/L or < 0.023 ng/dL. Values above the measuring range are reported as > 100 pmol/L or > 7.77 ng/dL.

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 0.300 pmol/L (0.023 ng/dL)

The lower detection limit represents the lowest analyte level that can be distinguished from 0.

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.

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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, pooled human sera and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	pmol/L	ng/dL	pmol/L	ng/dL	%	pmol/L	ng/dL	%
HS[b] 1	8.7	0.68	0.14	0.01	1.6	0.31	0.02	3.5
HS 2	21.1	1.64	0.35	0.03	1.7	0.71	0.06	3.3
HS 3	50.8	3.95	1.45	0.11	2.9	3.35	0.26	6.6
PC U[c]1	17.5	1.36	0.25	0.02	1.4	0.48	0.04	2.7
PC U2	26.1	2.03	0.48	0.04	1.8	0.79	0.06	3.0

b) HS = human serum

c) PC = PreciControl Universal

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Repeatability				
Sample	Mean		SD		CV
	pmol/L	ng/dL	pmol/L	ng/dL	%
HS 1	9.15	0.71	0.12	0.01	1.4
HS 2	16.9	1.31	0.30	0.02	1.8
HS 3	34.2	2.66	0.68	0.05	2.0
PC U1	11.4	0.89	0.16	0.01	1.4
PC U2	41.6	3.23	0.58	0.05	1.4

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Intermediate precision				
Sample	Mean		SD		CV
	pmol/L	ng/dL	pmol/L	ng/dL	%
HS 1	14.9	1.16	0.40	0.03	2.7
HS 2	17.5	1.36	0.46	0.04	2.6
HS 3	35.9	2.79	1.29	0.10	3.6
PC U1	11.9	0.92	0.32	0.02	2.7
PC U2	42.7	3.32	2.06	0.16	4.8

Method comparison

A comparison of the Elecsys FT4 assay (y) with the Enzymun-Test FT4 method (x) using clinical samples gave the following correlations (pmol/L):

Number of samples measured: 314

Passing/Bablok[8]	Linear regression
y = 1.03x + 0.39	y = 1.01x + 0.63
τ = 0.900	r = 0.987

The sample concentrations were between approx. 2 and 81 pmol/L (0.16 and 6.3 ng/dL).

Analytical specificity

For the antibody derivative used, the following cross-reactivities were found:

L-T4 and D-T4 100 %; L-T3 1.53 %; D-T3 1.38 %;
3-iodo-L-tyrosine 0.002 %; 3,5-diiodo-L-tyrosine 0.01 %;
3,3',5,5'-tetraiodothyroacetic acid 38.5 %.

References

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8 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, 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.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

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