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FT3

cobas®

FT3 - free triiodothyronine

REF	Σ	SYSTEM
03051986 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 triiodothyronine 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

Triiodothyronine is one of the thyroid hormones present in serum which regulates metabolism. Determination of this hormone concentration is important for the diagnostic differentiation of euthyroid, hyperthyroid, and hypothyroid states. The major fraction of total triiodothyronine is bound to the transport proteins (TBG, prealbumin, albumin). Free triiodothyronine (fT3) is the physiologically active form of the thyroid hormone triiodothyronine (T3).

The determination of free T3 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.[1,2,3]

The sequential testing procedure and the use of a labeled antibody reduces the possibility of interference due to altered binding properties of the serum, as can occur with assays employing labeled antigen (analog method).[4,5,6,7]

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.[4]

In the Elecsys FT3 test the determination of free triiodothyronine is made with the aid of a specific anti-T3 antibody labeled with a ruthenium complex[a)].

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 an anti-T3-specific antibody labeled with a ruthenium complex.
- 2nd incubation: After addition of biotinylated T3 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 is 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 FT3.

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

R1 Anti-T3-Ab~$Ru(bpy)_3^{2+}$ (gray cap), 1 bottle, 18 mL:
Monoclonal anti-T3-antibody (sheep) labeled with ruthenium complex 10 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 T3~biotin (black cap), 1 bottle, 18 mL:
Biotinylated T3 2 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	6 weeks

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 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, 1 month at -20 °C.[8] 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 03051994190, FT3 CalSet, for 4 x 1 mL
- REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- REF 11731416160, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2 (for USA)
- 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
- REF 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution (for USA)

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: The FT3 assay (REF 03051986) has been standardized against the FT3 assay (REF 11731386). This in turn was standardized using equilibrium dialysis.[9]

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, pg/mL or ng/dL).

Conversion factors:	pmol/L x 0.651 = pg/mL
	pg/mL x 1.536 = pmol/L
	pg/mL x 0.1 = ng/dL

Limitations - interference

The assay is unaffected by icterus (bilirubin < 564 µmol/L or < 33 mg/dL), hemolysis (Hb < 2.7 mmol/L or < 4.3 g/dL), lipemia (Intralipid < 2000 mg/dL), biotin (< 409 nmol/L or < 100 ng/mL), IgG < 7 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.

Of 18 commonly used pharmaceuticals tested in vitro, only furosemide caused increased fT3 findings at the daily therapeutic dosage level.

Any influence that might affect the binding behavior of the binding proteins can alter the result of the fT3 tests (e.g. drugs, NTIs (Non-Thyroid-Illness) or patients suffering from FDH (Familial Dysalbuminemic Hyperthyroxinemia)).[10]

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.[11]

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

0.400-50.0 pmol/L or 0.260-32.6 pg/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.400 pmol/L or < 0.260 pg/mL. Values above the measuring range are reported as > 50.0 pmol/L or > 32.6 pg/mL.

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 0.400 pmol/L or 0.260 pg/mL

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Samples for fT3 determinations cannot be diluted, as T3 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

Establishing values in reference range studies is based primarily on samples obtained from outpatient clinics, hospitals, and commercial laboratories in which TSH and fT4 levels are found to be in the euthyroid range.

These patients often have non-thyroid diseases which might influence the thyroid function in general, and especially the fT3 level.

This may explain the differences observed when comparing the reference ranges based on different population groups using the same fT3 method. Besides local differences in iodine intake the overall health status of the individuals involved is decisive for the outcome of the reference intervals.

The Elecsys FT3 assay was used to determine reference ranges in the following groups of individuals from different locations in Germany:

Adults:

• 3.1-6.8 pmol/L (2.0-4.4 pg/mL)

From a coastally-situated commercial laboratory in Germany, 5366 routine samples with TSH between 1 and 3 µIU/mL were evaluated by non-parametric calculation of the central 95 % limits and corresponding 95 % confidence intervals (CI) for fT3 concentration:

Median	2.5th percentile	95 % CI of the 2.5th percentile	97.5th percentile	95 % CI of the 97.5th percentile	Unit
4.6	3.1	3.07-3.19	6.8	6.65-6.87	pmol/L
3.0	2.0	2.00-2.08	4.4	4.33-4.47	pg/mL

• 3.9-6.7 pmol/L (2.5-4.3 pg/mL)

870 samples derived from apparently healthy blood donors aged 20 to 69 years from a central German site were evaluated by non-parametric calculation of the central 95 % limits and corresponding 95 % confidence intervals (CI) for fT3 concentration:

Median	2.5th percentile	95 % CI of the 2.5th percentile	97.5th percentile	95 % CI of the 97.5th percentile	Unit
5.1	3.9	3.67-3.99	6.7	6.54-7.00	pmol/L
3.3	2.5	2.39-2.60	4.3	4.26-4.56	pg/mL

The following parameters were recorded in these individuals: the concentration of TSH, fT4, and auto-antibodies to Tg and TPO; the volume and density of the thyroid gland measured by ultrasound; their case history, family, and personal thyroid history; their gender, age, and iodine intake; and whether or not they smoked or were taking oral contraceptives.

For results based on a variety of different inclusion and exclusion criteria, please refer to the separate information given in the brochure “Reference Intervals for Elecsys Thyroid Assays”.

• 2.4-6.3 pmol/L (1.5-4.1 pg/mL)

211 samples from dialysis patients were measured with the Elecsys FT3 assay in a multicenter evaluation (pilot study). The data represent the 2.5th and the 97.5th percentile.

• 1.3-6.3 pmol/L (0.8-4.1 pg/mL), median 3.2 pmol/L or 2.7 pg/mL

94 samples from patient suffering from severe non thyroid illnesses (NTI’s) were measured with the Elecsys FT3 assay in a multicenter evaluation (pilot study). The data represent the 2.5th and the 97.5th percentile.

Children and adolescence:

• Samples from newborns, infants, and adolescents up to 19 years of age, characterized as apparently healthy by experts from a medical center in central Germany:

Age	N	Median	2.5th percentile	95 % CI of the 2.5th percentile	Unit
4-30 days	40	5.2 3.4	3.0 2.0	2.6-3.8 1.7-2.5	pmol/L pg/mL
2-12 months	35	5.8 3.8	2.4 1.5	2.4-3.1 1.5-2.0	pmol/L pg/mL
2-6 years	73	6.1 4.0	3.0 2.0	2.9-3.6 1.9-2.4	pmol/L pg/mL
7-11 years	127	6.1 4.0	4.1 2.7	2.5-4.9 1.6-3.2	pmol/L pg/mL
12-19 years	140	5.9 3.9	3.5 2.3	3.1-3.7 2.0-2.4	pmol/L pg/mL

Age	N	Median	97.5th percentile	95 % CI of the 97.5th percentile	Unit
4-30 days	40	5.2 3.4	8.1 5.2	7.3-8.3 4.7-5.4	pmol/L pg/mL
2-12 months	35	5.8 3.8	9.8 6.4	8.8-9.8 5.7-6.4	pmol/L pg/mL
2-6 years	73	6.1 4.0	9.1 6.0	8.2-9.5 5.3-6.2	pmol/L pg/mL
7-11 years	127	6.1 4.0	7.9 5.2	7.6-9.2 5.0-6.0	pmol/L pg/mL
12-19 years	140	5.9 3.9	7.7 5.0	7.3-9.2 4.8-6.0	pmol/L pg/mL

The following exclusion criteria were stipulated in these individuals (both outpatient and hospitalized): no previous or acute thyroid disease, no family history of thyroid disease, no coronary disease, no intensive care, and no postoperative medical care.

Status: Adults: MCE, pilot study and β-site study Elecsys FT3 assay, REF 03051986, February 2004, and excerpt “MCE Reference Ranges Thyroid”, June 2004. Children and adolescence: Reference range study for thyroid values in children, June 2004.

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	pg/mL	pmol/L	pg/mL	%	pmol/L	pg/mL	%
HS[b)] 1	2.86	1.86	0.06	0.04	2.1	0.08	0.05	2.8
HS 2	3.85	2.51	0.08	0.05	2.2	0.10	0.07	2.7
HS 3	19.5	12.7	0.29	0.19	1.5	0.36	0.23	1.9
PC U[c)]1	4.98	3.24	0.08	0.05	1.7	0.11	0.07	2.2
PC U2	22.0	14.3	0.33	0.21	1.5	0.38	0.25	1.7

b) HS = human serum

c) PC U = PreciControl Universal

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Repeatability				
Sample	Mean		SD		CV
	pmol/L	pg/mL	pmol/L	pg/mL	%
HS 1	3.06	1.99	0.06	0.04	2.0
HS 2	4.15	2.70	0.08	0.05	2.0
HS 3	20.9	13.6	0.21	0.14	1.0
PC U1	4.92	3.20	0.09	0.06	1.9
PC U2	22.0	14.3	0.29	0.19	1.3

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Intermediate precision				
Sample	Mean		SD		CV
	pmol/L	pg/mL	pmol/L	pg/mL	%
HS 1	3.02	1.97	0.10	0.07	3.4
HS 2	4.04	2.63	0.10	0.07	2.5
HS 3	20.2	13.2	0.40	0.26	2.0
PC U1	5.04	3.28	0.13	0.08	2.6
PC U2	22.2	14.5	0.47	0.31	2.1

The following are the results obtained for the NCCLS (National Committee for Clinical Laboratory Standards) precision measurements in a laboratory in France in the course of the Multicenter Study for the Elecsys FT3 performance evaluation. These data represent the performance from routine measurements on the Elecsys 2010 analyzer in clinical centers.

Elecsys 2010 and **cobas e** 411 analyzers				
			Repeatability	**Intermediate precision**
Sample	Mean		CV	CV
	pmol/L	pg/mL	%	%
HS 1	2.83	1.84	3.3	5.1
HS 2	5.87	3.82	1.3	2.5
HS 3	15.5	10.1	2.2	3.7
PC U1	4.46	2.90	1.0	2.0
PC U2	22.8	14.8	1.5	2.1

Method comparison

A comparison of the FT3 assay, REF 03051986 (y) with the FT3 assay, REF 11731386 (x) using clinical samples gave the following correlations (pmol/L):

Number of samples measured: 964

Passing/Bablok[12]	Linear regression
y = 0.97x - 0.33	y = 1.0x - 0.44
τ = 0.810	r = 0.990

The sample concentrations were between approx. 0.74 and 48.5 pmol/L (approx. 0.48 and 31.6 pg/mL).

Analytical specificity

For the antibodies used, the following cross-reactivities were found:

L-T4 0.24 %; D-T4 0.40 %; L-rT3 n.d.[d)]; 3,5-diiodo-L-tyrosine 0.41 %; 3,3',5-triiodothyroacetic acid 58.2 %; 3,3',5,5'-tetra-iodothyroacetic acid 0.11 %.

d) n.d. = not detectable

References

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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.

FT3

FT3 - free triiodothyronine

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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