Elecsys FT3 III

cobas®

REF		Σ	SYSTEM
09005811190	09005811501	300	**cobas e** 402 **cobas e** 801

English

For use in the USA only

System information

Short name	ACN (application code number)
FT3 3	10220

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 **cobas e** immunoassay analyzers.

Summary

The thyroid hormones triiodothyronine (T3) and thyroxine (T4) are secreted into the bloodstream by the thyroid gland and play a vital role in regulating the body's metabolic rate, influencing the cardiovascular system, growth and bone metabolism, and are important for normal development of gonadal functions and nervous system.[1]

T3 circulates in the bloodstream as an equilibrium mixture of free and serum bound hormone. Free T3 (fT3) is the unbound and biologically active form, which represents only 0.2-0.4 % of the total T3. The remaining T3 is inactive and bound to serum proteins, while the distribution of T3 between these binding proteins (thyroxine binding globulin, pre-albumin, albumin) is controversially discussed.[2,3,4,5]

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. Therefore free T3 is a useful tool in clinical routine diagnostics for the assessment of the thyroid status. Free T3 measurements support the differential diagnosis of thyroid disorders, are needed to distinguish different forms of hyperthyroidism, and to identify patients with T3 thyrotoxicosis.[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 FT3 III assay a specific anti-T3 antibody labeled with a ruthenium complex[a)] is used to determine the free triiodothyronine concentration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 9 µL of sample and a 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 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 FT3 3.

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

R1 Anti-T3-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 19.7 mL:
Monoclonal anti-T3-antibody (sheep) labeled with ruthenium complex 18 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 T3~biotin, 1 bottle, 19.7 mL:
Biotinylated T3 2.4 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.

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 dust/fume/gas/mist/vapours/spray.
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.

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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.

Undiluted 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 ≤ ± 0.8 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 and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators 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 09077871190, FT3 III CalSet, for 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: This method has been standardized against the FT3 assay (REF 03051986190). The FT3 assay (REF 03051986190) is traceable to the FT3 assay (REF 11731386122) which was 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, 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 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	≤ 1128 µmol/L or ≤ 66 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

Criterion: Recovery of ± 0.4 pmol/L of initial value ≤ 4 pmol/L and ± 10 % of initial value > 4 pmol/L.

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)).[9,10]

Biotin interference

This assay has no biotin interference in serum concentrations up to 1200 ng/mL. 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 day[11] and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.[12]

Pharmaceutical substances
In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special thyroid drugs were tested. No interference with the assay was found.

Special thyroid drugs

Drug	Concentration tested µg/mL
Iodide	0.200
Carbimazole	30
Thiamazole	80
Propylthiouracil	60
Perchlorate	2000
Propranolol	240
Amiodarone	200
Prednisolone	100
Hydrocortisone	200
Fluocortolone	100
Octreotide	0.300

In in vitro studies the drugs furosemide, liothyronine and levothyroxine caused elevated fT3 findings at the daily therapeutic dosage level.

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

0.6-50 pmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.6 pmol/L. Values above the measuring range are reported as > 50 pmol/L.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.4 pmol/L

Limit of Detection = 0.6 pmol/L

Limit of Quantitation = 1.5 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 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.

- 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 perc.[b)]	95 % CI of the 2.5th percentile	97.5th perc.	95 % CI of the 97.5th percentile	Unit
4-30 days	40	5.2 3.4	3.0 2.0	2.6-3.8 1.7-2.5	8.1 5.2	7.3-8.3 4.7-5.4	pmol/L pg/mL
2-12 mos	35	5.8 3.8	2.4 1.5	2.4-3.1 1.5-2.0	9.8 6.4	8.8-9.8 5.7-6.4	pmol/L pg/mL
2-6 years	73	6.1 4.0	3.0 2.0	2.9-3.6 1.9-2.4	9.1 6.0	8.2-9.5 5.3-6.2	pmol/L pg/mL
7-11 years	127	6.1 4.0	4.1 2.7	2.5-4.9 1.6-3.2	7.9 5.2	7.6-9.2 5.0-6.0	pmol/L pg/mL

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Age	N	Median	2.5th perc.[b)]	95 % CI of the 2.5th percentile	97.5th perc.	95 % CI of the 97.5th percentile	Unit
12-19 years	140	5.9 3.9	3.5 2.3	3.1-3.7 2.0-2.4	7.7 5.0	7.3-9.2 4.8-6.0	pmol/L pg/mL

b) perc. = percentile

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.

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 (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 pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	1.62	0.0854	5.3	0.0862	5.3
Human serum 2	3.59	0.0956	2.7	0.102	2.8
Human serum 3	7.22	0.140	1.9	0.140	1.9
Human serum 4	25.6	0.321	1.3	0.335	1.3
Human serum 5	48.0	0.450	0.9	0.492	1.0
PreciControl U[c)]1	5.43	0.102	1.9	0.110	2.0
PreciControl U2	22.9	0.339	1.5	0.351	1.5

c) U = Universal

Method comparison

A comparison of the Elecsys FT3 III assay, REF 09005811190 (**cobas e** 801 analyzer; y) with the Elecsys FT3 III assay, REF 07027362190 (**cobas e** 801 analyzer; x) gave the following correlations (pmol/L):

Number of samples measured: 175

Passing/Bablok[13]	Linear regression
y = 1.04x - 0.061	y = 1.02x + 0.146
τ = 0.981	r = 0.998

The sample concentrations were between 1.48 and 49.5 pmol/L.

Analytical specificity

The following cross-reactivities were found, tested with fT3 concentrations of approximately 3.30 pmol/L (2.15 pg/mL) and 13.1 pmol/L (8.53 pg/mL):

Cross-reactant	Concentration tested pg/mL	Cross-reactivity %
L-T4	300000	0.008
D-T4	625000	0.005
rT3	10000000	0.000
3-iodo-L-tyrosine	100000000	0.000
3,5-diiodo-L-tyrosine	100000000	0.000
3,3',5-triiodothyroacetic acid	6250	0.282
3,3',5,5'-tetraiodothyroacetic acid	1000000	0.000

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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).

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

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