Elecsys T4

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REF		Σ	SYSTEM
09007784190* 09007784214*	09007784500	300	**cobas e** 402 **cobas e** 801

* Some kits shown may not be available in all countries.

English

System information

Short name	ACN (application code number)
T4	10120

Intended use

Immunoassay for the in vitro quantitative determination of 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

Total thyroxine (T4) measurements, performed with this assay, in human serum and plasma are used as an aid in the diagnosis of thyroid disorders.

Thyroid hormones are synthesized in the thyroid follicles whereas the dietary iodine is the basic element for their synthesis.[1] The thyroid glands secrete two hormones, thyroxine (T4) and triiodothyronine (T3), based on the number of iodine atoms in each molecule.[1] The thyroid hormones T3 and T4 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.[2]

T3 and T4 are secreted as free hormones into the bloodstream but as they are hydrophobic, they are almost completely bound to carrier proteins, the thyroxine-binding globulins (TBGs), prealbumin and albumin.[1] Total T4 is mostly composed of the bound version, only 0.03 % are free T4 (fT4).[1]

Total T4 measurement is a useful clinical diagnostic tool when the thyroid functions need to be tested for differentiating between euthyroid, hyperthyroid, and hypothyroid conditions.

As the concentrations of the transport proteins in serum are subject to endogenous and exogenous effects, the status of the binding proteins must also be taken into account in the assessment of the thyroid hormone concentration in serum. If this is ignored, changes in the binding proteins (e.g. due to the estrogen-containing preparations, during pregnancy or in the presence of a nephrotic syndrome etc) can lead to erroneous assessments of the thyroid metabolic state.[1,3,4,5,6] As the thyroid status correlates more with the free, rather than the total T4, the physician must also obtain fT4 concentration to aid in the detection of hyperthyroidism and hypothyroidism.[1,2,7]

The Elecsys T4 assay employs a competitive test principle with an antibody specifically directed against T4. Endogenous T4, released by the action of 8-anilino-1-naphthalene sulfonic acid (ANS), competes with the added biotinylated T4-derivative for the binding sites on the antibodies labeled with the 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: 9 µL of sample and a T4-specific antibody labeled with a ruthenium complex; bound T4 is released from binding proteins in the sample by ANS.
- 2nd incubation: After addition of streptavidin-coated microparticles and biotinylated T4, 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 T4.

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

R1 Anti-T4-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 19.7 mL:
Polyclonal anti-T4-antibody (sheep) labeled with ruthenium complex 100 ng/mL; ANS 1 mg/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

R2 T4~biotin, 1 bottle, 19.7 mL:
Biotinylated T4 20 ng/mL; phosphate buffer 100 mmol/L, pH 7.4; 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 available via the **cobas** link.

Elecsys T4

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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.

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.

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 ≤ ± 7.8 nmol/L + coefficient of correlation ≥ 0.95.

Stable for 4 days at 20-25 °C, 8 days at 2-8 °C, 12 months 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.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 12017717122, T4 CalSet, 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **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 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.

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: The Elecsys T4 assay has been checked by ID-GC/MS (isotope dilution gas chromatography mass spectrometry) on various control materials.[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

Use PreciControl Universal or other suitable controls for routine quality control procedures.

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 nmol/L, µg/dL or µg/L).

Conversion factors:
nmol/L x 0.077688 = µg/dL
µg/dL x 12.872 = nmol/L
nmol/L x 0.77688 = µg/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	≤ 633 µmol/L or ≤ 37 mg/dL
Hemoglobin	≤ 1.4 mmol/L or ≤ 2300 mg/dL
Intralipid	≤ 28.5 mmol/L or ≤ 2500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 2400 IU/mL

Criterion: For concentrations of 5.4-35 nmol/L the deviation is ≤ 3.5 nmol/L. For concentrations > 35 nmol/L the deviation is ≤ 10 %.

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

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

Special drugs

Drug	Concentration tested mg/L
Iodide	0.2

Elecsys T4

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Drug	Concentration tested mg/L
Carbimazole	30
Methimazole	48
Propylthiouracil	300
Perchlorate	2000
Propranolol	240
Amiodarone	40
Prednisolone	100
Hydrocortisone	200
Fluocortolone	100
Octreotide	0.3
Liothyronine	0.015

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.

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.[9]

Autoantibodies to thyroid hormones can interfere with the assay.

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

5.4-320 nmol/L or 0.420-24.86 µg/dL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 5.4 nmol/L or < 0.420 µg/dL. Values above the measuring range are reported as > 320 nmol/L or > 24.86 µg/dL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 5.0 nmol/L (0.388 µg/dL)

Limit of Detection = 5.4 nmol/L (0.420 µg/dL)

Limit of Quantitation = 15 nmol/L (1.17 µg/dL)

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 defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 20 %.

Dilution

Not necessary due to the broad measuring range.

Expected values

Measurements with the Elecsys T4 assay on 2526 serum samples from euthyroid test subjects in Europe and Japan yielded the following values (2.5th-97.5th percentile):
66-181 nmol/L or 5.1-14.1 µg/dL

FT4 Index (T4/TBI[b]) calculated from 825 serum samples from euthyroid test subjects in Europe and Japan measured with the Elecsys T4 assay and the Elecsys T-Uptake assay (2.5th-97.5th percentile):
62-164 nmol/L or 4.8-12.7 µg/dL

Measurements with the Elecsys T4 assay on 235 serum and plasma samples from euthyroid test subjects in the USA yielded the following values (0.5th-99.5th percentile):
58-155 nmol/L or 4.5-12.1 µg/dL

FT4 Index calculated from 233 serum and plasma samples from euthyroid test subjects in the USA measured with the Elecsys T4 assay and the Elecsys T-Uptake assay (0.5th-99.5th percentile):
55-139 nmol/L or 4.2-10.8 µg/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) TBI = Thyroxine-Binding Index

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 nmol/L	SD nmol/L	CV %	SD nmol/L	CV %
Human serum 1	6.58	0.217	3.3	0.472	7.2
Human serum 2	18.9	0.344	1.8	0.856	4.5
Human serum 3	53.5	1.46	2.7	2.38	4.4
Human serum 4	78.5	1.72	2.2	3.43	4.4
Human serum 5	175	4.29	2.5	6.76	3.9
Human serum 6	304	6.13	2.0	11.1	3.6
PC[c] Universal 1	101	1.82	1.8	3.84	3.8
PC Universal 2	151	2.61	1.7	5.35	3.5

c) PC = PreciControl

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean µg/dL	SD µg/dL	CV %	SD µg/dL	CV %
Human serum 1	0.511	0.017	3.3	0.037	7.2
Human serum 2	1.47	0.027	1.8	0.067	4.5
Human serum 3	4.16	0.113	2.7	0.185	4.4
Human serum 4	6.10	0.134	2.2	0.266	4.4
Human serum 5	13.6	0.333	2.5	0.525	3.9
Human serum 6	23.6	0.476	2.0	0.862	3.6
PC Universal 1	7.85	0.141	1.8	0.298	3.8
PC Universal 2	11.7	0.203	1.7	0.416	3.5

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Method comparison

a) A comparison of the Elecsys T4 assay, REF 09007784190 (**cobas e** 801 analyzer; y), with the Elecsys T4 assay, REF 07027885190 (**cobas e** 801 analyzer; x), gave the following correlations (nmol/L):

Number of samples measured: 219

Passing/Bablok[10]	Linear regression
y = 0.975x + 2.93	y = 0.955x + 5.04
τ = 0.967	r = 0.998

The sample concentrations were between 12.5 and 319 nmol/L.

b) A comparison of the Elecsys T4 assay, REF 09007784190 (**cobas e** 402 analyzer; y), with the Elecsys T4 assay, REF 09007784190 (**cobas e** 801 analyzer; x), gave the following correlations (nmol/L):

Number of samples measured: 219

Passing/Bablok[10]	Linear regression
y = 1.01x - 1.26	y = 1.00x - 0.795
τ = 0.970	r = 0.999

The sample concentrations were between 13.3 and 310 nmol/L.

Analytical specificity

The following cross-reactivities were found, tested with T4 concentrations of approximately 70 nmol/L and 160 nmol/L.

Cross-reactant	Cross-reactivity %
L-T3	not significant
T3	not significant
3-iodo-L-tyrosine	not significant
3,5-diiodo-L-tyrosine	0.004
3,3',5,5'-tetraiodothyroacetic acid	31.5
rT3	3.6
3,3',5-triiodothyroacetic acid	not significant

References

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10 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 user guide or 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 navifyportal.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
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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