

REF			SYSTEM
09007733190	09007733500	300	cobas e 402 cobas e 801

English

System information

Short name	ACN (application code number)
T3	10032

Intended use

Immunoassay for the in vitro quantitative determination of total triiodothyronine in human serum and plasma.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Triiodothyronine (3,5,3'-triiodothyronine or T3) is the thyroid hormone principally responsible for the regulation of metabolism of the various target organs.

T3 is mainly formed extrathyroidally, particularly in the liver, by enzymatic 5'-deiodination of T4 (thyroxine). Accordingly, the T3 concentration in serum is more a reflection of the functional state of the peripheral tissue than the secretory performance of the thyroid gland.¹

A reduction in the conversion of T4 to T3 results in a decrease in the T3 concentration. It occurs under the influence of medicaments such as propranolol, glucocorticoids or amiodarone and in severe non-thyroidal illness (NTI), and is referred to as "low T3 syndrome". As with T4, over 99 % of T3 is bound to transport proteins. However, the affinity of T3 to them is around 10-fold lower, but T3 has a 15-fold higher affinity for thyroid receptor compared to T4.^{1,2,3}

The determination of T3 is utilized in the diagnosis of T3-hyperthyroidism, the detection of early stages of hyperthyroidism and for indicating a diagnosis of thyrotoxicosis factitia.^{2,4,5,6}

The Elecsys T3 assay employs a competitive test principle with polyclonal antibodies specifically directed against T3. Endogenous T3, released by the action of 8-anilino-1-naphthalene sulfonic acid (ANS), competes with the added biotinylated T3-derivative for the binding sites on the antibodies labeled with the ruthenium complex^{a)}.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 18 µL of sample and a T3-specific antibody labeled with a ruthenium complex; bound T3 is released from the binding proteins in the sample by ANS.
- 2nd incubation: After addition of streptavidin-coated microparticles and biotinylated T3, 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 T3.

- M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-T3 Ab~Ru(bpy)₃²⁺, 1 bottle, 18.8 mL:
Polyclonal anti-T3-antibody (sheep) labeled with ruthenium complex 75 ng/mL; ANS 0.8 mg/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

- R2 T3~biotin, 1 bottle, 18.8 mL:
Biotinylated T3 3 ng/mL; ANS 0.8 mg/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 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: 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.

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, K₂-EDTA and K₃-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within ± 0.4 nmol/L + coefficient of correlation ≥ 0.95 .

Stable for 8 days at 20-25 °C, 14 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] 11731548122, T3 CalSet, for 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: This method has been standardized against reference standards by weighing T3 into analyte-free human serum matrix.

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 nmol/L, ng/mL or ng/dL).

Conversion factors:

nmol/L x 0.651 = ng/mL
nmol/L x 65.09998 = ng/dL
ng/mL x 1.536 = nmol/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	≤ 599 $\mu\text{mol/L}$ or ≤ 35 mg/dL
Hemoglobin	≤ 1.2 mmol/L or ≤ 2000 mg/dL
Intralipid	≤ 1800 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1500 IU/mL

Criterion: For concentrations of 0.3-2 nmol/L the deviation is ≤ 0.2 nmol/L. For concentrations > 2 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
Carbimazole	30
Methimazole	80
Perchlorate	2000
Propranolol	48
Amiodarone	200
Prednisolone	100
Hydrocortisone	200
Fluocortolone	100
Octreotide	0.3

In in vitro studies the drug propylthiouracil caused decreased T3 findings at the daily therapeutic dosage level.

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.

Different medications can affect T3 levels.⁷ For example, therapy with amiodarone can lead to depressed T3 values. Phenytoin, phenylbutazone, and salicylates cause release of T3 from the binding proteins, thus leading to a reduction in the total T3 hormone level at normal fT3 levels.⁸

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

Pathological concentrations of binding proteins (TBG, albumin) can lead to total T3 values outside the normal range being found despite a euthyroid metabolic state (e.g. in NTI^b) patients, pregnancy, use of oral contraceptives). In such cases a fT3 or fT4 determination is indicated.

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.

b) NTI = non thyroidal illness

Limits and ranges

Measuring range

0.3-10 nmol/L or 0.195-6.51 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.3 nmol/L or < 0.195 ng/mL. Values above the measuring range are reported as > 10 nmol/L or > 6.51 ng/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.2 nmol/L (0.130 ng/mL)

Limit of Detection = 0.3 nmol/L (0.195 ng/mL)

Limit of Quantitation = 0.4 nmol/L (0.260 ng/mL)

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

Not necessary due to the broad measuring range.

Expected values

1.3-3.1 nmol/L or 0.8-2.0 ng/mL: euthyroid

The values correspond to the 2.5th and 97.5th percentiles of findings from a total of 514 healthy subjects.

Status: MCE Elecsys 2010, 1996

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.

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	0.510	0.0239	4.7	0.0331	6.5
Human serum 2	1.32	0.0330	2.5	0.0403	3.1
Human serum 3	3.42	0.0622	1.8	0.0728	2.1
Human serum 4	5.64	0.0930	1.7	0.121	2.1
Human serum 5	8.76	0.129	1.5	0.165	1.9
PC ^{c)} Universal 1	2.54	0.0427	1.7	0.0490	1.9
PC Universal 2	5.64	0.0822	1.5	0.0931	1.7

c) PC = PreciControl

Method comparison

a) A comparison of the Elecsys T3 assay, [REF] 09007733190 (cobas e 801 analyzer; y), with the Elecsys T3 assay, [REF] 07027869190 (cobas e 801 analyzer; x), gave the following correlations (nmol/L):

Number of samples measured: 126

Passing/Bablok ¹⁰	Linear regression
$y = 0.964x + 0.118$	$y = 0.944x + 0.186$
$\tau = 0.962$	$r = 0.998$

The sample concentrations were between 0.304 and 9.83 nmol/L.

b) A comparison of the Elecsys T3 assay, [REF] 09007733190 (cobas e 402 analyzer; y), with the Elecsys T3 assay, [REF] 09007733190 (cobas e 801 analyzer; x), gave the following correlations (nmol/L):

Number of samples measured: 154

Passing/Bablok ¹⁰	Linear regression
$y = 1.01x - 0.014$	$y = 1.06x - 0.113$
$\tau = 0.975$	$r = 0.999$

The sample concentrations were between 0.319 and 9.28 nmol/L.

Analytical specificity

The following cross-reactivities were found, tested with a T3 concentration of approximately 1.8 nmol/L.

Cross-reactant	Concentration tested	Cross-reactivity %
L-T4	620 ng/mL	0.527
D-T4	620 ng/mL	0.520
rT3	10 µg/mL	0.012
L-T2	10000 µg/dL	< 0.001
3,3',5-triiodothyroacetic acid	0.249 µg/dL	77.0
3,3',5,5'-tetraiodothyroacetic acid	100 µg/dL	0.312

References

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- 4 Surks MI, Chopra IJ, Mariash CN, et al. American Thyroid Association guidelines for use of laboratory tests in thyroid disorders. JAMA 1990;63:1529-1532.
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- 9 Wada N, Chiba H, Shimizu C, et al. A Novel Missense Mutation in Codon 218 of the Albumin Gene in a Distinct Phenotype of Familial Dysalbuminemic Hyperthyroxinemia in a Japanese Kindred. Journal of Clinical Endocrinology and Metabolism 1997;82(10):3246-3250.
- 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 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.

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):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume after reconstitution or mixing
	Global Trade Item Number



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