06368590500V11.0

Elecsys Anti-TPO

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REF		Σ	SYSTEM
06368590190	06368590500	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 720
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 137

Intended use

Immunoassay for the in vitro quantitative determination of antibodies to thyroid peroxidase in human serum and plasma. The anti-TPO determination is used as an aid in the diagnosis of autoimmune thyroid diseases.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Thyroid-specific peroxidase (TPO) is synthesized in the endoplasmic reticulum, where it is folded to its native state and undergoes core glycosylation, before being transported to the apical plasma membrane of thyrocytes.[1,2]

In synergy with thyroglobulin (Tg) this enzyme has an essential function in the iodination of L-tyrosine and the chemical coupling of the resulting mono- and di-iodotyrosine to form the thyroid hormones T4, T3, and rT3.[3]

TPO is a potential autoantigen. Elevated serum titers of antibodies to TPO are found in several forms of thyroiditis caused by autoimmunity.[4,5] TPO was identified as the causative antigen in 1985 when studies demonstrated that human antisera reacting to "microsomal antigen" precipitated TPO prepared from Graves' disease thyroid tissue.[6,7] Clinically the two terms anti-TPO and microsomal antibody can be used synonymously; there are differences, however, with regard to the test methods.

High anti-TPO titers are found in up to 90 % of patients with chronic Hashimoto's thyroiditis. In Graves' disease, 70 % of the patients have an elevated titer.[4,8,9] Although the sensitivity of the procedure can be increased by simultaneously determining other thyroid antibodies (anti-Tg, TSH-receptor-antibody - TRAb), a negative finding does not rule out the possibility of an autoimmune disease. The magnitude of the antibody titer does not correlate with the clinical activity of the disease.[8,9,10] Initially elevated titers can become negative after lengthy periods of illness or during remission. If antibodies reappear following remission, then a relapse is probable.[11]

Whereas the usual microsomal antibody tests employ unpurified microsomes as an antigen preparation, the anti-TPO tests use a purified peroxidase. The two procedures are of comparable performance in terms of clinical sensitivity, but better lot-to-lot consistency and higher clinical specificity can be expected from anti-TPO tests due to the higher quality of the antigen used.

Recombinant antigen and polyclonal anti-TPO antibodies are used in the Elecsys Anti-TPO assay.

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample are incubated with anti-TPO-antibodies labeled with a ruthenium complex[a)].
- 2nd incubation: After addition of biotinylated TPO and streptavidin-coated microparticles, the anti-TPO antibodies in the sample compete with the ruthenium-labeled anti-TPO antibodies for the biotinylated TPO antigen. 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 or e-barcode.

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

Reagents - working solutions

The reagent rackpack is labeled as A-TPO.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-TPO-Ab~$Ru(bpy)_3^{2+}$ (gray cap), 1 bottle, 9 mL:
Polyclonal anti-TPO antibody (sheep) labeled with ruthenium complex 1.0 mg/L; TRIS buffer 100 mmol/L, pH 7.2; preservative.

R2 TPO~biotin (black cap), 1 bottle, 9 mL:
Biotinylated TPO (recombinant) 0.15 mg/L; TRIS buffer 30 mmol/L, pH 7.0; preservative.

Precautions and warnings

For in vitro diagnostic use for laboratory 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.

Hazardous components:

- 2-methyl-2H-isothiazol-3-one hydrochloride

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).

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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	6 weeks
on the analyzers	2 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 plasma.

Criterion: Slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation ≥ 0.95.

Stable for 3 days at 2-8 °C, 1 month at -20 °C. Freeze only once.[12]

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 06472931190, Anti-TPO CalSet, for 4 x 1.5 mL
- REF 05042666191, PreciControl ThyroAB, for 4 x 2.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent
- REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **cobas e** 411 analyzer:

- 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, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **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, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- 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. 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 approximately 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 NIBSC (National Institute for Biological Standards and Control) 66/387 Standard.

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: Perform calibration on all analyzers as follows:

- with every reagent kit

Renewed calibration on all analyzers:

- daily: when using the same reagent kit on the analyzers
- as required: e.g. quality control findings outside the defined limits

Quality control

Use PreciControl ThyroAB 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 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.

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 IU/mL or kIU/L).

Limitations - interference

The assay is unaffected by icterus (bilirubin ≤ 1129 µmol/L or ≤ 66 mg/dL), hemolysis (Hb ≤ 0.15 mmol/L or ≤ 0.24 g/dL), lipemia (triglycerides ≤ 23.9 mmol/L or ≤ 2100 mg/dL) and biotin (≤ 40.9 nmol/L or ≤ 10 ng/mL).

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 450 IU/mL.

Pharmaceutical substances
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

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

Special drugs

Drug	Concentration tested µg/mL
Iodide	0.040
Carbimazole	6.00
Methimazole	16.0
Propylthiouracil	60.0
Perchlorate	400
Propranolol	48.0
Amiodarone	40.0
Prednisolone	20.0
Hydrocortisone	40.0
Fluocortolone	20.0
Octreotide	0.060
Levothyroxine	0.143
Liothyronine	0.015

In in vitro studies the drug itraconazol caused increased anti-TPO concentration 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

5.00-600 IU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 5.00 IU/mL. Values above the measuring range are reported as > 600 IU/mL.

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: < 5.00 IU/mL

The lower detection limit represents the lowest 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 with anti-TPO concentrations above the measuring range can be diluted manually with Diluent Universal. The recommended dilution is 1:5. The concentration of the diluted sample must be > 200 IU/mL. After dilution, multiply the result by the dilution factor.

Please note: The autoantibodies are heterogeneous and this gives rise to non-linear dilution phenomena for individual samples.

Expected values

Extended studies with the Elecsys Anti-TPO assay performed on samples from 208 healthy test subjects in 3 clinical centers in Austria and Germany showed a borderline value of 34 IU/mL for 95 % of the results.

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

cobas e 411 analyzer						
	Repeatability			**Intermediate precision**		
Sample	Mean IU/mL	SD IU/mL	CV %	Mean IU/mL	SD IU/mL	CV %
Human serum 1	15.3	1.07	7.0	12.4	3.02	24.4
Human serum 2	113	2.88	2.5	109	10.1	9.2
Human serum 3	269	11.4	4.2	308	21.9	7.1

cobas e 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean IU/mL	SD IU/mL	CV %	Mean IU/mL	SD IU/mL	CV %
Human serum 1	21.3	1.34	6.3	20.8	1.97	9.5
Human serum 2	51.2	2.61	5.1	53.1	3.25	6.1
Human serum 3	473	12.7	2.7	455	19.1	4.2

Precision was determined using Elecsys reagents and controls in a protocol (EP5-A2) 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 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
PC[b)] THYRO1	38.6	2.41	6.2	3.44	8.9
PC THYRO2	111	4.48	4.0	6.22	5.6

b) PC = PreciControl

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
PC THYRO1	37.2	1.78	4.8	2.27	6.1
PC THYRO2	106	2.98	2.8	3.77	3.5

Method comparison

A comparison of the Elecsys Anti-TPO assay (y) with a commercially available anti-TPO test (x) using clinical samples gave the following correlations:

Number of samples measured: 50

Passing/Bablok[13]	Linear regression
y = 0.77x + 2.95	y = 0.63x + 17.2
τ = 0.785	r = 0.899

The sample concentrations were between 12 and 460 IU/mL.

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Analytical specificity

A 0.3 % cross-reactivity with human autoantibodies to thyroglobulin (4000 IU/mL) were found, tested with anti-TPO concentrations of approximately 50 IU/mL and 250 IU/mL.

References

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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:

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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