

# Anti-TPO

Antibodies to thyroid peroxidase

cobas®

REF		SYSTEM
06368590 190	100	Elecsys 2010 MODULAR ANALYTICS E170 <b>cobas e 411</b> <b>cobas e 601</b> <b>cobas e 602</b>

## English

### 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 electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

### Summary

Thyroid-specific peroxidase (TPO) is present on the microsomes of thyrocytes and is expressed at its apical cell surface. 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.<sup>1</sup>

TPO is a potential autoantigen. Elevated serum titers of antibodies to TPO are found in several forms of thyroiditis caused by autoimmunity.<sup>2</sup> The still frequently found term "microsomal antibody" originates from the time when TPO had not yet been identified as an antigen in autoimmunity caused by microsomes. In the clinical sense 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. 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.<sup>3,4,5</sup> Initially elevated titers can become negative after lengthy periods of illness or during remission. If antibodies reappear following remission, then a relapse is probable.<sup>6</sup>

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<sup>a)</sup>.
- 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.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)<sub>3</sub><sup>2+</sup>)

### 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)<sub>3</sub><sup>2+</sup> (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.

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	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-, Na-, NH<sub>4</sub><sup>+</sup>-heparin, K<sub>3</sub>-EDTA, sodium citrate and sodium fluoride/potassium oxalate plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within  $\pm 2 \times$  analytical sensitivity (LDL) + coefficient of correlation  $> 0.95$ .

Stable for 3 days at 2-8 °C, at least 1 month at -20 °C. Freeze only once.<sup>7</sup>

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.



# Anti-TPO

Antibodies to thyroid peroxidase

**cobas**<sup>®</sup>

## 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 2 x 2 mL each of PreciControl ThyroAB 1 and 2
- [REF] 11732277122, Diluent Universal, 2 x 16 mL sample diluent
- [REF] 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- 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: 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

For quality control, use PreciControl ThyroAB.

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 IU/mL or kIU/L).

## Limitations - interference

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 0.93 mmol/L or < 1.5 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 1500 IU/mL.

In vitro tests were performed on 23 commonly used pharmaceuticals. No interference with the assay was found.

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, 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).



# Anti-TPO

## Antibodies to thyroid peroxidase

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 (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:

Elecsys 2010 and cobas e 411 analyzers						
Sample	Repeatability			Intermediate precision		
	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
PC A-TPO <sup>b</sup> 1	25.9	1.45	5.6	25.4	3.35	13.2
PC A-TPO2	112	3.78	3.4	116	8.83	7.6

b) PC A-TPO = PreciControl Anti-TPO

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers						
Sample	Repeatability			Intermediate precision		
	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
PC A-TPO1	19.4	1.07	5.5	22.0	2.02	9.2
PC A-TPO2	100	2.57	2.6	103	4.39	4.3

### 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<sup>8</sup> Linear regression

$$y = 0.77x + 2.94$$

$$r = 0.785$$

$$y = 0.63x + 17.1$$

$$r = 0.899$$

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

### Analytical specificity

No influence with human autoantibodies to thyroglobulin (< 394 IU/mL) was detectable.

### References

- 1 Pfannenstiel P, Saller B. Schilddrüsenkrankheiten – Diagnose und Therapie, 2nd edition. Berliner Medizinische Verlagsanstalt. 1995;28-30,141,169-172,200-201.
- 2 McIntosh RS, Asghar MS, Weetman AP. The antibody response in human autoimmune thyroid disease. Clin Sci 1997;(92):529-541.
- 3 Volpé R. Rational Use of Thyroid Function Tests. Crit Rev Clin Lab Sci 1997;34(5):405-438.
- 4 Feldt-Rasmussen U. Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin, and thyrotropin receptor. Clin Chem 1996;42(1):160-163.
- 5 Utiger RD. The pathogenesis of autoimmune thyroid disease. N Eng J Med 1991;325:278-279.

- 6 Gutekunst R. Hashimoto-Thyreoiditis: Diagnostik und Verlaufskontrolle. In: Börner W, Weinheimer B (Hrsg.): Schilddrüse 1989. Walter de Gruyter, Berlin, New York 1991;348-355.
- 7 Greiling H, Gressner AM. Lehrbuch der Klinischen Chemie und Pathobiochemie. 3rd edition, Stuttgart; New York: Schattauer 1995:1012.
- 8 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.

### 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 after reconstitution or mixing

### FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

COBAS, COBAS E, ELECSYS, MODULAR and PRECICONTROL are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

Significant additions or changes are indicated by a change bar in the margin.

© 2013, Roche Diagnostics



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim  
www.roche.com

Distribution in USA by:  
Roche Diagnostics, Indianapolis, IN  
US Customer Technical Support 1-800-428-2336

