**Appendix (3)**

**Thyroxine**

**REF** 200 tests  
12017 709 122

- Indicates analyzers on which the kit can be used

<table>
<thead>
<tr>
<th>cobas e 602</th>
<th>cobas e 601</th>
<th>cobas e 411</th>
<th>MODULAR ANALYZERS</th>
<th>Elecsys 2010</th>
</tr>
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<tbody>
<tr>
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**English**

**Intended use**
Immunossay for the in vitro quantitative determination of thyroxine in human serum and plasma.

The electrochemiluminescence immunossay “ECLA” is intended for use on Elecsys and cobas e immunossay analyzers.

**Summary**

The hormone thyroxine (T4) is the main product secreted by the thyroid gland and is an integral component of the hypothalamus-anterior pituitary-thyroid hormone regulating system. It has the function of anabolically influencing metabolism. Thyroxine is found in a reaction of two DIT molecules (3,5-diiodothyrosine) in the thyroid gland. It is stored bound to thyroglobulin in the lumina of the thyroid follicles and is secreted as required under the influence of TSH. The major part (> 99%) of total thyroxine (T4) in serum is present in protein-bound form. As the concentrations of the transport proteins in serum are subject to exogenous and endogenous 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 estrogens, pregnancy, or in the presence of a nephrotic syndrome etc.) can lead to erroneous assessments of the thyroid metabolic state.

The determination of T4 can be utilized for the following indications: the detection of hypothyroidism, the detection of primary and secondary hypothyroidism, and the monitoring of TSH-suppression therapy.

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 Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy) ) is formed.

**Test principle**

**Competition principle. Total duration of assay: 18 minutes.**

1. Incubation: 15 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.

2. 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.

3. 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. Application of a volt age 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.

**Reagents - working solutions**

**M** Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL; Streptavidin-coated microparticles 0.72 ng/mL; preservative.

**R1** Anti-T4-Ab/Ru(bpy)  (gray cap), 1 bottle, 18 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 (black cap), 1 bottle, 18 mL: Biotinylated T4 20 ng/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.

**Pr ecautions and warnings**

- **For in vitro diagnostic use.**
- Exercise the normal precautions required for handling all reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

**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 via the respective reagent barcodes.

**Storage and stability**

Store at 2-8 °C.

Store the Elecsys T4 reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use. Stability:

- up to the stated expiration date: Opened at 2-8 °C
- 12 weeks after opening at 2-8 °C
- 8 weeks on the analyzers

**Specimen collection and preparation**

Only the specimens listed below were tested.

- Serum collected using standard sampling tubes or tubes containing separating gel.
- Li+, Na-heparin, K3-EDTA, and sodium citrate plasma.
- Sodium fluoride/potassium oxalate are used, the values found are by approx. 26% lower than those for serum.
- Stable for 7 days at 2-8 °C, 30 days at -20 °C. Freeze only once.

**Materials provided**

See “Reagents - working solutions” section for reagents.

**Materials required (but not provided)**

- 12017 711722, T4 CalSet, 4 x 1 mL
- 111731416122, PreciControl Univer sal, for 2 x 3 mL each of PreciControl Univer sal 1 and 2 or 111731416190, PreciControl Univer sal, for 2 x 3 mL each of PreciControl Univer sal 1 and 2
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYZERS E170 or cobas e analyzer

**Accessories for Elecsys 2010 and cobas e 411 analyzers:**

- 11662988122, ProCell, 6 x 380 mL system buffer
- 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- 11933159001, Adapter for SysClean
Thyroxine

- **REF** 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- **REF** 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTIC TICS E170, cobas e 601 and cobas e 602 analyzers:
- **REF** 4880340190, ProCell M, 2 x 2 L system buffer
- **REF** 4880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- **REF** 03023141001, PC/CC-Cups, 12 cups to prewar m ProCell M and CleanCell M before use
- **REF** 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run initialization and rinsing during reagent change
- **REF** 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 8 reaction vessels or pipette tips, waste bags
- **REF** 03022350001, WasteLiner, waste bags
- **REF** 03027651001, SysClean Adapter M

Accessories for all analyzers:
- **REF** 11298500316, 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 before use. Read in the test-specific parameter via the reagent barcode. In exceptional cases the bar code 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 the formation of foam. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

**Calibration**

Traceability: The Elecsys T4 test has been checked by ID-GC/MS (isotope dilution gas chromatography mass spectrometry) on various control materials. Every Elecsys T4 reagent set has a bar-coded label containing the specific inter matrix for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys T4 CalSet. Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Renewed calibration is recommended as follows:
- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the specified limits

**Quality control**

For quality control, use Elecsys PreciControl Univer sal sal 1 and 2. Other suitable control material can be used in addition. Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and at every calibration. The control inter vals 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 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 nmol/L, µg/dL, ng/L).

Converter factor: $nmol/L \times 0.077688 = \mu g/dL$

$\mu g/dL \times 12.872 = nmol/L$

$nmol/L \times 0.77688 = \mu g/L$

**Limitations - inter ference**

The assay is unaffected by icterus (bilirubin < 633 µmol/L or < 37 mg/dL), hemolysis (Hb < 1.4 mmol/L or < 2.3 g/dL), lipemia (triglycerides < 28.5 mmol/L or < 2500 mg/dL), and biotin < 409 nmol/L or < 100 ng/mL.

**Criterion:** Recover y within ± 10 % of initial value.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 10 hours after the last biotin administration.

N o inter ference was observed from rheumatoid factor s up to a concentration of 2400 IU/mL and samples from dialysis patients. In vitro tests were performed on 15 commonly used pharmaceuticals. N o inter ference with the assay was found.

The test cannot be used in patients receiving treatment with lipid-lowering agents containing D-T4. If the thyroid function is to be checked in such patients, the therapy should first be discontinued for 4-6 weeks to allow the physiological state to become re-established.

Autoantibodies to thyroid hormone can interfere with the assay. 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.

In rare cases, interference due to extremely high titer s 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**

Lower detection limit: 5.40 nmol/L (0.420 µg/dL)

Lower limits of measurement

Lower detection limit: 5.40 nmol/L (0.420 µg/dL)

The detection limit represents the lowest measurable 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**

N ot necessary due to the broad measuring range.

**Expected values**

Measurements with the Elecsys T4 assay on 2526 serum samples from euthyroid test subjects in Germany and Japan yielded the following values (2.5th-97.5th percentile):

- 66-181 nmol/L or 2.1-14.1 µg/dL
- FT4 Index (T4/TBI) calculated from 825 serum samples from euthyroid test subjects measured with the Elecsys T4 assay and the Elecsys T-Uptake assay (2.5th-97.5th percentile):
- 62-16 4 nmol/L or 4.8-12.7 µg/dL

Following values were determined for the 99 % percentile range from 275 serum and plasma samples from healthy test subjects in USA:

- 59-15 4 nmol/L or 4.6-12.0 µg/dL

**FT4 Index:** 57-147 nmol/L or 4.4-11.4 µg/dL

For detailed information about reference intervals in children, adolescents and pregnant women, refer to the brochure “Reference intervals for children and adults.”

**Accessories for all analyzers:**

- **REF** 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

<table>
<thead>
<tr>
<th>Elecsys and cobas e analyzers</th>
<th>vi</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Properties:</strong></td>
<td></td>
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<tr>
<td><strong>Clinical chemistry:</strong></td>
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<td><strong>Reference intervals:</strong></td>
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<td><strong>Assay description:</strong></td>
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<td><strong>Technical information:</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Appendices:</strong></td>
<td></td>
</tr>
</tbody>
</table>

**cobas®**
Specific performance data

Representative performer data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EPS-A) of the CLSI (Clinical and Laboratory Standards Institute). Six times daily for 10 days (n = 60); repeatability on the MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

<table>
<thead>
<tr>
<th>CV</th>
<th>SD (µg/dL)</th>
<th>µmol/L</th>
<th>CV</th>
<th>SD (µg/dL)</th>
<th>µmol/L</th>
<th>Mean (µg/dL)</th>
<th>µmol/L</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.9</td>
<td>0.18</td>
<td>2.37</td>
<td>4.7</td>
<td>0.12</td>
<td>1.58</td>
<td>2.59</td>
<td>33.4</td>
<td>HS^1</td>
</tr>
<tr>
<td>3.7</td>
<td>0.35</td>
<td>4.56</td>
<td>2.7</td>
<td>0.26</td>
<td>3.38</td>
<td>9.59</td>
<td>123</td>
<td>HS^2</td>
</tr>
<tr>
<td>3.0</td>
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<td>237</td>
<td>HS^3</td>
</tr>
<tr>
<td>3.3</td>
<td>0.29</td>
<td>3.78</td>
<td>2.3</td>
<td>0.20</td>
<td>2.54</td>
<td>8.79</td>
<td>115</td>
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<tr>
<td>2.7</td>
<td>0.28</td>
<td>4.90</td>
<td>2.0</td>
<td>0.28</td>
<td>3.58</td>
<td>14.0</td>
<td>181</td>
<td>PC U^2</td>
</tr>
</tbody>
</table>

b) Repeatability = within-run precision
c) HS = human serum
d) PC U = PreciControl Universal

<table>
<thead>
<tr>
<th>CV</th>
<th>SD (µg/dL)</th>
<th>µmol/L</th>
<th>Mean (µg/dL)</th>
<th>µmol/L</th>
<th>Sample</th>
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<tbody>
<tr>
<td>3.7</td>
<td>0.19</td>
<td>2.40</td>
<td>5.09</td>
<td>65.6</td>
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<td>3.3</td>
<td>0.49</td>
<td>6.29</td>
<td>14.7</td>
<td>190</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Method comparison

A comparison of the Elecsys T4 assay (y) with the Enzymun Test T4 method (x) using clinical samples gave the following correlations (nmol/L): Number of samples measured: 71

Linear regression

\[ y = 0.75x + 9.88 \]
\[ r = 0.975 \]
\[ \tau = 0.841 \]

The sample concentrations were between 8 and 250 nmol/L (0.6 and 19 µg/dL).

Analytical specificity

For the antibody derivative used, the following cross-reactivities were found: L-T4 and D-T4 100%; L-T3 1.53%; D-T3 1.38%; 3,5-diido-L-tyrosine 0.002%; 3,5-diido-L-tyrosine 0.01%; 3,3’,5’,5’-tetraiodothyroacetic acid 38.5%.

References


For further information, please refer to the appropriate operator’s manual for the analyzer concerned, the respective application sheets, the product information, and the package inserts of all necessary components.

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