**Elecsys FT4 III**

<table>
<thead>
<tr>
<th>REF</th>
<th>SYSTEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>07976836 190</td>
<td>MODULAR ANALYTICS E170</td>
</tr>
<tr>
<td></td>
<td>cobas e 411</td>
</tr>
<tr>
<td></td>
<td>cobas e 601</td>
</tr>
<tr>
<td></td>
<td>cobas e 602</td>
</tr>
</tbody>
</table>

**English**

**System information**
For **cobas e 411** analyzer: test number 1610
For **MODULAR ANALYTICS E170, cobas e 601** and **cobas e 602** analyzers: Application Code Number 309

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

The electrochemiluminescence immunocassay “ECLIA” is intended for use on Elecsys and cobas e immunoassay analyzers.

**Summary**
Thyroxine (T4) is the main thyroid hormone secreted into the bloodstream by the thyroid gland. Together with triiodothyronine (T3) it plays a vital role in regulating the body’s metabolic rate, influences the cardiovascular system, growth and bone metabolism, and is important for normal development of gonadal functions and nervous system. T4 circulates in the bloodstream as an equilibrium mixture of free and serum bound hormone. Free T4 (fT4) is the unbound and biologically active form, which represents only 0.03 % of the total T4. The remaining T4 is inactive and bound to serum proteins such as thyroxine binding globulin (TBG) (75 %), pre-albumin (15 %), and albumin (10 %). The determination of free T4 has the advantage of being independent of changes in the concentrations and binding properties of these binding proteins; additional determination of a binding parameter (T-uptake, TBG) is therefore unnecessary. Thus free T4 is a useful tool in clinical routine diagnostics for the assessment of the thyroid status. It should be measured together with TSH if thyroid disorders are suspected and is also suitable for monitoring thyro depressive therapy. A variety of methods are available for estimating the free thyroid hormone levels. The direct measurement of T4 and T3 via equilibrium dialysis or ultrafiltration is mainly used as a reference method for standardizing, the immunological procedures generally used for routine diagnostic purposes.

In the Elecsys FT4 III assay a specific anti-T4 antibody labeled with a ruthenium complex is used to determine the free thyroxine.

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

**Test principle**

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

- 1st incubation: 15 µL of sample and a T4-specific antibody labeled with a ruthenium complex.
- 2nd incubation: After addition of biotinylated T4 and streptavidin-coated microparticles, 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/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 which is instrument-specific generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

**Reagents - working solutions**
The reagent rackpack is labeled as FT4 III.

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

| R1 | Anti-T4-Ab–Ru(bpy)²⁺ (gray cap), 1 bottle, 18 mL: Polyclonal anti-T4-antibody (sheep) labeled with ruthenium complex 75 ng/mL; phosphate buffer 100 mmol/L, pH 7.0; preservative. |
| R2 | T4–biotin (black cap), 1 bottle, 18 mL: Biotinylated T4 2.5 ng/mL; phosphate buffer 100 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.

<table>
<thead>
<tr>
<th>Stability:</th>
<th>up to the stated expiration date</th>
</tr>
</thead>
<tbody>
<tr>
<td>unopened at 2-8 °C</td>
<td>up to the stated expiration date</td>
</tr>
<tr>
<td>after opening at 2-8 °C</td>
<td>84 days</td>
</tr>
<tr>
<td>on the analyzers</td>
<td>28 days onboard or 56 days when stored alternatively in the refrigerator and on the analyzer, with the total time onboard on the analyzer not exceeding 120 hours</td>
</tr>
</tbody>
</table>

**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 ± 0.6 pmol/L + coefficient of correlation ≥ 0.95.

Stable for 5 days at 20-25 °C, 7 days at 2-8 °C, 30 days 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.
**Elecsys FT4 III**

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)**
- 07976879190, CalSet FT4 III, 4 x 1.0 mL
- 11731416190, PreciControl Universal, for 4 x 3.0 mL
- General laboratory equipment
- MODULAR ANALYTICS E170 or cobas e analyzer

Accessories for cobas e 411 analyzer:
- 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
- 11706802001, AssayCup, 60 x 60 reaction cups
- 11706799001, AssayTip, 30 x 120 pipette tips
- 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers:
- 04880340190, ProCell M, 2 x 2 L system buffer
- 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- 03023150001, WasteLiner, waste bags
- 03027651001, SysClean Adapter M

Accessories for all analyzers:
- 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 (except for the cobas e 602 analyzer).

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers: PreClean M solution is necessary. 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 Elecsys FT4 II method. The Elecsys FT4 II assay is traceable to the Enzymun-Test which was standardized using equilibrium dialysis.5,6,8 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:** 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).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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 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 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 pmol/L, ng/dL or ng/L.

Conversion factors:
- pmol/L x 0.77688 = ng/dL
- ng/dL x 12.872 = pmol/L
- pmol/L x 0.77688 = ng/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**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>≤ 701 pmol/L or ≤ 41 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>≤ 0.621 mmol/L or ≤ 1000 ng/dL</td>
</tr>
<tr>
<td>Intra lipid</td>
<td>≤ 2000 mg/dL</td>
</tr>
<tr>
<td>Biotin</td>
<td>≤ 409 nmol/L or ≤ 100 ng/mL</td>
</tr>
<tr>
<td>Rheumatoid factors</td>
<td>≤ 1200 IU/mL</td>
</tr>
<tr>
<td>IgG</td>
<td>≤ 7 g/dL</td>
</tr>
<tr>
<td>IgA</td>
<td>≤ 1.6 g/dL</td>
</tr>
<tr>
<td>IgM</td>
<td>≤ 1 g/dL</td>
</tr>
</tbody>
</table>

Criterion: Recovery of ≤ ± 0.6 pmol/L of initial value ≤ 6 pmol/L and ± 10 % of initial value > 6 pmol/L.

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.

Any influence that might affect the binding behavior of the binding proteins can alter the result of the FT4 tests (e.g. drugs, NTIs (Non-Thyroid-Illness) or patients suffering from FDH (Familial Dysalbuminemic Hyperthyroxinemia)).5,8,10

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.11

Autoantibodies to thyroid hormones can interfere with the assay.7

**Pharmaceutical substances**

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

In addition, the following special thyroid drugs were tested. No interference with the assay was found.
Elecys FT4 III

Special thyroid drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration tested µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodide</td>
<td>0.2</td>
</tr>
<tr>
<td>Carbimazole</td>
<td>6</td>
</tr>
<tr>
<td>Thiamazole</td>
<td>80</td>
</tr>
<tr>
<td>Propylthioureacl</td>
<td>300</td>
</tr>
<tr>
<td>Perchlorate</td>
<td>2000</td>
</tr>
<tr>
<td>Propranolol</td>
<td>240</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>200</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>100</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>200</td>
</tr>
<tr>
<td>Flucortolone</td>
<td>100</td>
</tr>
<tr>
<td>Octreotide</td>
<td>0.3</td>
</tr>
</tbody>
</table>

In in vitro studies the drugs Furosemide and Levothryoxine caused elevated FT4 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

0.5-100 pmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.5 pmol/L. Values above the measuring range are reported as > 100 pmol/L.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.3 pmol/L
Limit of Detection = 0.5 pmol/L
Limit of Quantitation = 1.5 pmol/L

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 the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples for FT4 determinations cannot be diluted, as T4 in the blood is present in free and protein-bound forms which are in equilibrium. A change in the concentration of the binding proteins alters this equilibrium.

Expected values

Euthyroid: 12-22 pmol/L (0.93-1.7 ng/dL)

These values correspond to the 2.5th and 97.5th percentile of results from a total of 601 healthy test subjects studied.

Status: MCE Reference Range Thyroid, Status 1st quarter 1998.

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 04629388.

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 for 21 days (n = 84). The following results were obtained:

<table>
<thead>
<tr>
<th>cobas e 411 analyzer</th>
<th>Repeatability</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Mean pmol/L</td>
<td>SD pmol/L</td>
</tr>
<tr>
<td>Human serum 1</td>
<td>1.55</td>
<td>0.089</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>13.6</td>
<td>0.239</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>23.9</td>
<td>0.411</td>
</tr>
<tr>
<td>Human serum 4</td>
<td>57.8</td>
<td>1.11</td>
</tr>
<tr>
<td>Human serum 5</td>
<td>92.0</td>
<td>2.24</td>
</tr>
<tr>
<td>PreciControl U1</td>
<td>15.8</td>
<td>0.258</td>
</tr>
<tr>
<td>PreciControl U2</td>
<td>41.0</td>
<td>0.867</td>
</tr>
</tbody>
</table>

b) U = Universal

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repeatability</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean pmol/L</td>
<td>SD pmol/L</td>
</tr>
<tr>
<td>Human serum 1</td>
<td>1.63</td>
<td>0.070</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>13.0</td>
<td>0.198</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>22.7</td>
<td>0.348</td>
</tr>
<tr>
<td>Human serum 4</td>
<td>54.7</td>
<td>0.925</td>
</tr>
<tr>
<td>Human serum 5</td>
<td>92.7</td>
<td>2.37</td>
</tr>
<tr>
<td>PreciControl U1</td>
<td>15.3</td>
<td>0.173</td>
</tr>
<tr>
<td>PreciControl U2</td>
<td>39.0</td>
<td>0.695</td>
</tr>
</tbody>
</table>

Method comparison

A comparison of the Elecsys FT4 III assay (y) with the Elecsys FT4 II assay (x) using clinical samples gave the following correlations:

Number of samples measured: 141

$y = 1.03x + 0.178$  
$y = 1.02x + 0.610$  
$r = 0.999$

The sample concentrations were between approximately 2.3 and 92 pmol/L.

Analytical specificity

The following cross-reactivities were found, tested with FT4 concentrations of approximately 13 pmol/L and 39 pmol/L:

<table>
<thead>
<tr>
<th>Cross-reactant</th>
<th>Concentration tested ng/dL</th>
<th>Cross-reactivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-T3</td>
<td>50000</td>
<td>0.005</td>
</tr>
<tr>
<td>D-T3</td>
<td>50000</td>
<td>0.002</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Cross-reactant</th>
<th>Concentration tested ng/dL</th>
<th>Cross-reactivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>rT3</td>
<td>190000</td>
<td>0.007</td>
</tr>
<tr>
<td>3-iodo-L-tyrosine</td>
<td>10000000</td>
<td>0.000</td>
</tr>
<tr>
<td>3,5-diiodo-L-tyrosine</td>
<td>1000000</td>
<td>0.000</td>
</tr>
<tr>
<td>3',5'-triiodothyroacetic acid</td>
<td>100000</td>
<td>0.000</td>
</tr>
<tr>
<td>3',5'-tetraiodothyroacetic acid</td>
<td>100000</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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 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 (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

- **CONTENT**: Contents of kit
- **SYSTEM**: Analyzers/Instruments on which reagents can be used
- **REAGENT**: Reagent
- **CALIBRATOR**: Calibrator
- **STIN**: Volume after reconstitution or mixing
- **GTIN**: Global Trade Item Number

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