Elecsys Prolactin II

**System information**

For cobas e 411 analyzer: test number 131
For cobas e 601 and cobas e 602 analyzers: Application Code Number 014

**Intended use**

Immunocassay for the in vitro quantitative determination of prolactin in human serum and plasma.

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

**Summary**

Prolactin is synthesized in the anterior pituitary and is secreted in episodes. The hormone is made up of 198 amino acids and has a molecular weight of approximately 22-23 kDa. Prolactin appears in serum in three different forms. The biologically and immunologically active monomeric ("little") form predominates, followed by the biologically inactive dimeric ("big") form and the tetrameric ("big-big") form having low biological activity.\(^1,2\) The target organ for prolactin is the mammary gland, the development and differentiation of which is promoted by this hormone. High concentrations of prolactin have an inhibiting action on steroidogenesis of the ovaries and on hypothalamic gonadotropin production and secretion. During pregnancy the concentration of prolactin rises under the influence of elevated estrogen and progesterone production. The stimulating action of prolactin on the mammary gland leads postpartum to lactation. Prolactin further affects glucose and lipid metabolism and may be involved in the manifestation of insulin resistance.\(^3,4,5\)

Hyperprolactinemia (in men and women) is a cause of fertility disorders.\(^6\) The determination of prolactin is utilized in the diagnosis of hyperprolactinemia\(^7,8\) and peritoneal endometriosis.\(^9\)

The Elecsys Prolactin II assay uses two monoclonal antibodies specifically directed against human prolactin.\(^10\)

Both antibodies show a low reactivity with most forms of macroprolactin.

**Test principle**

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample and a biotinylated monoclonal prolactin-specific antibody form a first complex.
- 2nd incubation: After addition of a monoclonal prolactin-specific antibody labeled with a ruthenium complex\(^10\) and streptavidin-coated microparticles, a sandwich complex is formed and 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)\(^{2+}\))

**Reagents - working solutions**

The reagent rackpack is labeled as PRL II.

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
  Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-prolactin-Ab–biotin (gray cap), 1 bottle, 10 mL:
  Biotinylated monoclonal anti-prolactin antibody (mouse) 0.7 mg/mL; phosphate buffer 50 mmol/L; pH 7.0; preservative.

**Precautions and warnings**

For in vitro diagnostic use for healthcare 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:

| R2 | Anti-prolactin-Ab–Ru(bpy)\(^{2+}\) (black cap), 1 bottle, 10 mL: Monoclonal anti-prolactin antibody (mouse) labeled with ruthenium complex 0.35 mg/L; phosphate buffer 50 mmol/L, pH 7.0; preservative.

**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 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:**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time</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>12 weeks</td>
</tr>
<tr>
<td>On the analyzers</td>
<td>8 weeks</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.

**Criterion:** Slope: 0.9-1.1 + intercept within \( \pm 10 \mu U/mL \) + coefficient of correlation \( \geq 0.95 \).

Stable for 5 days at 20-25 °C, 14 days at 2-8 °C, 6 months at -20 °C (± 5 °C). Freeze only once.

Stability of serum obtained with separating tubes: 24 hours at 2-8 °C (note the data provided by the tube manufacturer).

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 several 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)**

- 03277356190, Prolactin II CalSet, for 4 x 1.0 mL
- 11731416190, PreciControl Universal, for 4 x 3.0 mL
- 11732277122, Diluent Universal, 2 x 16 mL sample diluent or 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas** e 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 waterwash additive
- 11933159001, Adapter for SysClean
- 11706802001, AssayCup, 60 x 60 reaction cups
- 11706799001, AssayTip, 30 x 120 pipette tips
- 11800507001, Clean-Liner

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

- 11930346122, Elecsys SysWash, 1 x 500 mL waterwash additive
- 11933159001, Adapter for SysClean
- 11706802001, AssayCup, 60 x 60 reaction cups
- 11706799001, AssayTip, 30 x 120 pipette tips
- 11800507001, Clean-Liner

Additional materials for the **cobas** e 601 and **cobas** e 602 analyzers:

- 04880304190, ProCell M, 2 x 2 L system buffer
- 04880293190, ProCell 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 solution cleaning setup for run initialization and rinsing during reagent change
- 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- 03023150001, WasteLiner, waste bags
- 03027651001, SysClean Adapter M

**Additional materials 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.

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 3rd IRP WHO Reference Standard 84/500.

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 µU/mL, ng/mL or in mIU/L).

Conversion factors:

\[ \mu U/mL \times 0.047 = ng/mL \]

\[ ng/mL \times 21.2 = \mu U/mL \]

**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>≤ 513 µmol/L or ≤ 30 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>≤ 0.932 mmol/L or ≤ 1500 mg/dL</td>
</tr>
<tr>
<td>Intraalbumin</td>
<td>≤ 1500 mg/dL</td>
</tr>
<tr>
<td>Biotin</td>
<td>≤ 164 nmol/L or ≤ 40 ng/mL</td>
</tr>
<tr>
<td>Rheumatoid factors</td>
<td>≤ 1100 IU/mL</td>
</tr>
</tbody>
</table>
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Criterion: For concentrations of 1-50 μIU/mL, the deviation is ± 10 μIU/mL. For concentrations > 50-100 μIU/mL, the deviation is ±20%. For concentrations > 100 μIU/mL, the deviation is ±50%.

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.

There is no high-dose hook effect at prolactin concentrations up to 270000 μIU/mL (12690 ng/mL).

Pharmacological substances
In vitro tests were performed on 16 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.

When determining prolactin it should be remembered that the measured concentration is dependent upon when the blood sample was taken, since the secretion of prolactin occurs in episodes and is also subject to a 24-hour cycle.

The release of prolactin is inhibited by dopamine, L-dopa and ergotamine derivatives.

A number of publications report the presence of macroprolactin in the serum of female patients with various endocrinological diseases or during pregnancy. Differing degrees of detection of the serum macroprolactins relative to monomeric prolactin (22-23 kDa) by various immunoassays have also been described. This could lead to a false diagnosis of hyperprolactinemia depending on the immunoassay used.

In case of implausible high prolactin values a precipitation by polyethylene glycol (PEG) is recommended in order to estimate the amount of the biological active monomeric prolactin.

See section “Sample pretreatment by polyethylene glycol (PEG) precipitation” for further details.

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
1.00-10000 μIU/mL or 0.0470-470 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 1 μIU/mL or < 0.0470 ng/mL. Values above the measuring range are reported as > 10000 μIU/mL or > 470 ng/mL (or up to 100000 μIU/mL or 4700 ng/mL for 10-fold diluted samples).

Lower limits of measurement
Lower detection limit of the test
Lower detection limit: 1.00 μIU/mL (0.047 ng/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 prolactin concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:10 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 50 μIU/mL or > 2.4 ng/mL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values
A study with the Elecsys Prolactin II assay was performed using samples from 300 apparently healthy blood donors. The following results were obtained:

<table>
<thead>
<tr>
<th>Percentiles</th>
<th>50th</th>
<th>2.5-97.5th</th>
<th>50th</th>
<th>2.5-97.5th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>μIU/mL</td>
<td>ng/mL</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>102</td>
<td>155</td>
<td>86-324</td>
<td>7.30</td>
</tr>
</tbody>
</table>

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 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:

<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</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>μIU/mL</td>
<td>ng/mL</td>
</tr>
<tr>
<td>HS1</td>
<td>10.2</td>
<td>0.48</td>
</tr>
<tr>
<td>HS 2</td>
<td>74.9</td>
<td>3.52</td>
</tr>
<tr>
<td>HS 3</td>
<td>567</td>
<td>26.6</td>
</tr>
<tr>
<td>HS 4</td>
<td>5333</td>
<td>199</td>
</tr>
<tr>
<td>HS 5</td>
<td>8083</td>
<td>266</td>
</tr>
<tr>
<td>PC U01</td>
<td>243</td>
<td>11.4</td>
</tr>
<tr>
<td>PC U2</td>
<td>859</td>
<td>40.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>cobas e 601 and cobas e 602 analyzers</th>
<th>Repeatability</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td></td>
<td>μIU/mL</td>
<td>ng/mL</td>
</tr>
<tr>
<td>HS 1</td>
<td>9.96</td>
<td>0.47</td>
</tr>
<tr>
<td>HS 2</td>
<td>71.6</td>
<td>3.37</td>
</tr>
<tr>
<td>HS 3</td>
<td>5233</td>
<td>246</td>
</tr>
<tr>
<td>HS 4</td>
<td>529</td>
<td>24.9</td>
</tr>
<tr>
<td>PC U1</td>
<td>229</td>
<td>10.8</td>
</tr>
<tr>
<td>PC U2</td>
<td>806</td>
<td>37.9</td>
</tr>
</tbody>
</table>

Method comparison
A comparison of the Elecsys Prolactin II assay (y) with the Elecsys Prolactin assay (x) using clinical samples containing no significant amounts of macroprolactin gave the following correlations (μIU/mL):

Number of samples measured: 227

Passing/Bablok

Regression

y = 0.74x - 10.36

r = 0.998

The sample concentrations were between 10 and 9063 μIU/mL (0.47 and 426 ng/mL).
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Analytical specificity
The monoclonal antibodies used are highly specific against prolactin. No cross reaction with hGH, hCG, hPL, TSH, FSH and LH has been observed.

Sample pretreatment by polyethylene glycol (PEG) precipitation

Test principle
Macroprolactin and oligomers can be precipitated by using a 25 % aqueous PEG solution (ratio 1+1). After centrifugation, the supernatant containing monomeric prolactin is used in the Elecsys Prolactin II assay in the same way as a native sample. The dilution effect which occurs during sample pretreatment and the coprecipitation of monomeric prolactin must be taken into consideration.

Reagents (not provided)
- Polyethylene glycol 6000 (e.g. available from Serva, Cat. No. 33137)
- Distilled or deionized water

Precautions and warnings
See instructions provided by the manufacturer of the polyethylene glycol 6000.

Reagent handling
To prepare a 25 % PEG solution, dissolve 25 g polyethylene glycol 6000 in approximately 60 mL of distilled or deionized water at 18-25 °C (magnetic stirrer, 15 minutes) and fill up to 100 mL.

Storage and stability
Store the original substance according to the instructions of the manufacturer. Store the 25 % PEG solution at 20-25 °C. Stability of the solution: 7 days.

Materials required (but not provided)
- Magnetic stirrer
- Rotating shaker (vortex)
- Centrifuge (1500 g to 10000 g)

Assay
Sample pretreatment (18-25 °C):
- Mix appropriate volume of sample (at least 180 µL) with PEG solution at a ratio of 1+1
- Mix well for approximately 10 seconds in a rotating shaker (vortex)
- Centrifuge for 5 minutes between 1500 g and 10000 g (within 1-30 minutes)

Analyze the supernatant in the same way as the native samples.

Calculation
Approximately 14 % (range: 0-40 %) of monomeric prolactin is coprecipitated by PEG. The dilution effect which occurs during PEG treatment and the coprecipitation of monomeric prolactin must be taken into consideration when calculating the results.

After precipitation by PEG each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

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.

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):

CONTENT
Contents of kit
SYSTEM
Analyzers/Instruments on which reagents can be used
REAGENT
Reagent
CALIBRATOR
Calibrator
STIM
Volume after reconstitution or mixing

Global Trade Item Number

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