

Prolactin II

cobas®

REF	Σ	SYSTEM
03203093 190	100	MODULAR ANALYTICS E170 cobas e 411 cobas e 601 cobas e 602

For USA: Elecsys Prolactin Assay

English

System-Information

For **cobas e 411** analyzer: test number 131
For MODULAR ANALYTICS E170, **cobas e 601** and **cobas e 602** analyzers: Application Code Number 014

Intended use

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

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay 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 kD. Prolactin appears in serum in three different forms. The biologically and immunologically active monomeric ("little") form predominates (approximately 80 %), 5-20 % is present as the biologically inactive dimeric ("big") form and 0.5-5 % is present as the tetrameric ("big-big") form having low biological activity.¹ 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 hypophyseal 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 post partum to lactation.

Hyperprolactinemia (in men and women) is the main cause of fertility disorders. The determination of prolactin is utilized in the diagnosis of anovular cycles, hyperprolactinemic amenorrhea and galactorrhea, gynecomastia and azoo-spermia. Prolactin is also determined when breast cancer and pituitary tumors are suspected.²

The Elecsys Prolactin II assay uses two monoclonal antibodies specifically directed against human prolactin.

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^{a)} 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)₃²⁺)

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/L;
phosphate buffer 50 mmol/L, pH 7.0; preservative.

- R2 Anti-prolactin-Ab~Ru(bpy)₃²⁺ (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.

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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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	12 weeks
on the analyzers	8 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 and K₃-EDTA plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 3 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 14 days at 2-8 °C, 6 months at -20 °C. Freeze only once.³

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 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.

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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] 03277356190, Prolactin II CalSet, for 4 x 1 mL
- [REF] 11731416190, PreciControl Universal, for 4 x 3 mL
- [REF] 11731416160, PreciControl Universal, for 4 x 3 mL (for USA)
- [REF] 11732277122, Diluent Universal, 2 x 16 mL sample diluent or [REF] 03183971122, Diluent Universal, 2 x 36 mL sample diluent

- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **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

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, 48 magazines x 84 reaction cups or pipette tips, waste bags
- [REF] 03023150001, WasteLiner, waste bags
- [REF] 03027651001, SysClean Adapter M

Accessories for all analyzers:

- [REF] 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution
- [REF] 11298500160, ISE Cleaning Solution/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 (except for the **cobas e** 602 analyzer).

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 $\mu\text{IU/mL}$, ng/mL or in mIU/L).

Conversion factors: $\mu\text{IU/mL (mIU/L)} \times 0.047 = \text{ng/mL}$
 $\text{ng/mL} \times 21.2 = \mu\text{IU/mL (mIU/L)}$

Limitations - interference

The assay is unaffected by icterus (bilirubin < 513 $\mu\text{mol/L}$ or < 30 mg/dL), hemolysis (Hb < 0.932 mmol/L or < 1.5 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 164 nmol/L or < 40 ng/mL).

Criterion: Recovery within $\pm 15\%$ 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 approximately 1100 IU/mL .

There is no high-dose hook effect at prolactin concentrations up to 270000 $\mu\text{IU/mL}$ (12690 ng/mL).

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 promoted physiologically by suckling and stress. In addition, elevated serum prolactin concentrations are caused by a number of pharmaceuticals (e.g. dibenzodiazepines, phenothiazine), TRH and estrogen.^{4,5,6}

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.^{1,7,8} Differing degrees of detection of the serum macroprolactins relative to monomeric prolactin (22-23 kD) by various immunoassays have also been described. This could make the detection of hyperprolactinemia dependent on the immunoassay used.^{7,9,10}

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.^{11,12,13,9,14,15,16,10,17,18}

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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:

Percentiles					
		50 th	2.5-97.5 th	50 th	2.5-97.5 th
	N	µIU/mL		ng/mL	
Men	102	155	86-324	7.30	4.04-15.2
Women (not-pregnant)	198	225	102-496	10.6	4.79-23.3

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

cobas e 411 analyzer								
Sample	Mean		Repeatability			Intermediate precision		
			SD		CV	SD		CV
	µIU/mL	ng/mL	µIU/mL	ng/mL	%	µIU/mL	ng/mL	%
HS ^{b)} 1	300	14.1	11.9	0.559	4.0	14.9	0.700	5.0
HS 2	718	33.7	15.5	0.728	2.2	25.9	1.22	3.6
HS 3	2700	127	49.0	2.30	1.8	75.3	3.54	2.8
HS 4	4945	232	96.4	4.53	2.0	182	8.55	3.7
PC U ^{c)} 1	154	7.24	5.0	0.235	3.3	6.51	0.306	4.2

cobas e 411 analyzer								
Sample	Mean		Repeatability			Intermediate precision		
			SD		CV	SD		CV
	µIU/mL	ng/mL	µIU/mL	ng/mL	%	µIU/mL	ng/mL	%
PC U2	582	27.3	15.4	0.724	2.6	19.5	0.916	3.4

b) HS = human serum

c) PC U = PreciControl Universal

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
Sample	Repeatability				
	Mean		SD		CV
	µIU/mL	ng/mL	µIU/mL	ng/mL	%
HS 1	182	8.55	1.47	0.069	0.8
HS 2	598	28.1	10.2	0.479	1.7
HS 3	2314	109	26.3	1.24	1.1
PC U1	162	7.61	1.44	0.068	0.9
PC U2	613	28.8	5.10	0.240	0.8

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
Sample	Intermediate precision				
	Mean		SD		CV
	µIU/mL	ng/mL	µIU/mL	ng/mL	%
HS 1	288	13.5	5.17	0.243	1.8
HS 2	871	40.9	12.0	0.564	1.4
HS 3	4477	210	69.5	3.27	1.6
PC U1	167	7.85	2.28	0.107	1.4
PC U2	634	29.8	12.9	0.606	2.0

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/Bablok¹⁹ Linear regression

y = 0.74x - 10.36

y = 0.76x - 21.21

r = 0.942

r = 0.998

The sample concentrations were between 10 and 9063 µIU/mL (0.47 and 426 ng/mL).

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

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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.¹⁵ 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.

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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):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume after reconstitution or mixing
	Global Trade Item Number

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