Elecsys Progesterone III

English

System Information

| REF              | 07027699190 |
|------------------|-------------|
| **Short name**   | PROG 3      |
| **ACN**          | 10045       |

**Intended use**

Immunoenzymometric assay for the in vitro quantitative determination of progesterone in human serum and plasma. The electrochemiluminescence immunoassay “ECLIA” is intended for use on the cobas e 801 immunoassay analyzer.

**Summary**

The gestagen progesterone is a steroid hormone which is mainly formed in the cells of the corpus luteum and during pregnancy in the placenta.

The progesterone concentration correlates with the development and regression of the corpus luteum. Whereas progesterone is barely detectable in the follicular phase of the female cycle, a rise in the progesterone level is observed one day prior to ovulation. Increased progesterone synthesis occurs during the luteal phase. In the second half of the pregnancy, progesterone is secreted in urine as the main degradation product of progesterone. 

Progesterone brings about the conversion of the uterine mucosa into a tissue rich in glands (secretion phase), in order to prepare for the intruterine implantation of the fertilized ovum. During pregnancy, progesterone inhibits the contraction of the myometrium. In the mammary gland, progesterone (together with estrogen) promotes the proliferation, secretion and disposition of the alveoli.

The determination of progesterone is utilized in fertility diagnosis for the detection of ovulation and assessment of the luteal phase.

**Test principle**

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating the sample (12 µL) with a progesterone-specific biotinylated antibody, immunocomplexes are formed, the amount of which is dependent upon the analyte concentration in the sample.
- 2nd incubation: After addition of streptavidin-coated microparticles and an progesterone derivative labeled with a ruthenium complex, the still-vacant sites of the biotinylated antibodies 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 II 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 cobas link.

a) Tris(2,2′-bipyridyl)ruthenium[II] complex (Ru(bpy)₂⁺)

**Reagents - working solutions**

The cobas e pack is labeled as PROG 3.

- **M** Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
  Streptavidin-coated microparticles 0.72 mg/mL; preservative.

- **R1** Anti-progesterone-Ab-biotin, 1 bottle, 21.0 mL:
  Biotinylated monoclonal anti-progesterone antibody (recombinant, sheep) 30 ng/mL; phosphate buffer 25 mM, pH 7.0; preservative.

- **R2** Progesterone-peptide-Ru(bpy)₂⁺, 1 bottle, 18.8 mL:
  Progesterone (of vegetable origin) coupled to a synthetic peptide labeled with ruthenium complex, 2 ng/mL; phosphate buffer 25 mM, 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 available via the cobas link.

**Storage and stability**

Store at 2-8 °C.

Do not freeze.

Store the cobas e pack 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
- on the cobas e 801 analyzer 16 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, K₃-EDTA and K₂-EDTA plasma.

Criterion: Slope 0.9-1.1 + intercept within ± 0.1 ng/mL + coefficient of correlation ≥ 0.95.

- Stable for 1 day at 20-25 °C, 5 days at 2-8 °C, 6 months at -20 °C (± 5 °C).
- Freeze only once.

The samples tested 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 specimens and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators 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 07092547190, Progesterone III CalSet, for 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3 mL
- REF 03028542122, Diluent Estradiol/Progesterone, 2 x 22 mL sample diluent
- General laboratory equipment
- cobas e 801 analyzer

**Accessories for the cobas e 801 analyzer:**

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cups, 8 cups to supply ProCell II M and CleanCell M
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- 06908653190, PreClean II M, 2 x 2 L wash solution
- 05694302001, Assay Tip/Assay Cup tray, 6 magazines
- 74900917001, 105 assay tips and 105 assay cups, 3 wasteliners
- 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply
- 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply
- 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. Place the cooled (stored at 2–8 °C) cobas e pack on the reagent manager. Avoid foaming formation. The system automatically regulates the temperature of the reagents and the opening/closing of the cobas e pack.

Calibration
Traceability: The Elecsys Progesterone III assay is traceable via ID-GC/MS (isotope dilution gas chromatography/mass spectrometry) to highly purified progesterone by weight analogously to BCR-348R and ERM-DA347.6

The pre-defined 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 cobas e pack 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 8 weeks when using the same reagent lot
- after 28 days when using the same cobas e pack 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 cobas e pack, 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 nmol/L, mg/mL or in µg/L).

Conversion factors:

| Conversion factor | Unit |
|------------------|------|
| nmol/L x 0.314   | ng/mL |
| ng/mL x 3.18     | nmol/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

| Compound  | Concentration tested |
|-----------|----------------------|
| Bilirubin | ≤ 923 µmol/L or ≤ 54 mg/dL |
| Hemoglobin | ≤ 0.621 mmol/L or ≤ 1000 mg/dL |

### Drug substances

| Drug                  | Concentration tested mg/L |
|-----------------------|---------------------------|
| Clomiphene citrate    | 100                       |

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**
0.159-191 nmol/L or 0.05-60 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.159 nmol/L or < 0.05 ng/mL. Values above the measuring range are reported as > 191 nmol/L or > 60 ng/mL.

**Lower limits of measurement**

Limit of Blank, Limit of Detection and Limit of Quantitation

| Limit of Blank | Limit of Detection | Limit of Quantitation |
|----------------|--------------------|-----------------------|
| 0.080 nmol/L (0.025 ng/mL) | 0.159 nmol/L (0.05 ng/mL) | 0.636 nmol/L (0.2 ng/mL) |

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 defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 20 %.

### Dilution

Samples with progesterone concentrations above the measuring range can be diluted with Diluent Estradiol/Progesterone or a suitable human serum with a low analyte concentration. The recommended dilution is 1:10. The concentration of the diluted sample must be ≥ 3.18 nmol/L (≥ 1 ng/mL).

After manual dilution, multiply the result by the dilution factor.
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Depending on the biological variance of the diluted patient sample and the human serum matrix used for production of Diluent Estradiol/Progesterone, lower recovery of diluted samples may be observed.

**Expected values**
The expected ranges were determined by testing specimens drawn from 147 apparently healthy males, 142 apparently healthy, post-menopausal women over the age of 50, and from 416 apparently healthy pregnant women between the ages of 17 and 45 (137 in the first trimester, 140 in the second trimester, and 139 in the third trimester). The expected range for healthy women was determined by weekly blood drawing over a period of 3 months from 26 apparently healthy women between the ages of 18 and 45 that were not taking any hormonal contraceptives. Based on a central 90 % interval, the following ranges were obtained:

| Test subjects | N | 5th percentile | Median | 95th percentile |
|---------------|---|----------------|--------|-----------------|
| Healthy men   | 147 | < 0.159 | < 0.159 | 0.474 |
| Healthy women |     |           |        |                 |
| Follicular phase | 117 | 0.181 | 0.588 | 2.84 |
| Ovulation phase | 38 | 0.385 | 1.60 | 38.1 |
| Luteal phase | 126 | 5.82 | 31.9 | 75.9 |
| Postmenopause | 142 | < 0.159 | < 0.159 | 0.401 |
| Healthy pregnant women | | | | |
| 1st trimester | 137 | 35.0 | 76.3 | 141 |
| 2nd trimester | 140 | 80.8 | 151 | 264 |
| 3rd trimester | 139 | 187 | 340 | 681 |

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 analyzer is 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:

| Sample | Mean | SD | CV |
|--------|------|----|----|
| Human serum 1 | 0.172 | 0.054 | 0.035 | 0.011 | 20.7 |
| Human serum 2 | 2.10 | 0.659 | 0.089 | 0.028 | 4.2 |
| Human serum 3 | 9.64 | 3.03 | 0.264 | 0.083 | 2.7 |

**Method comparison**
A comparison of the Elecsys Progesterone III assay, REF. 07027699190 (cobas e 801 analyzer; y) with the Elecsys Progesterone III assay, REF. 07092539190 (cobas e 601 analyzer; x) gave the following correlations (ng/mL):

- Mean: 153
- Passing/Bablok:
  - Linear regression: $y = 0.984x + 0.001$
  - $r = 0.999$

The sample concentrations were between 0.050 and 59.0 ng/mL.

**Analytical specificity**
For the Elecsys Progesterone III assay, the following cross-reactivities were found at the respective additive concentration, tested with progesterone concentrations of approximately 0.3 ng/mL and 5 ng/mL:

| Substance | Additive concentration ng/mL | Cross-reactivity % |
|-----------|-----------------------------|--------------------|
| Androstenediol | 4000 | 0.001 |
| Androstenedione | 80 | 0.107 |
| Aldosterone | 1000 | 0.003 |
| Allopregnanolone | 2000 | 0.347 |
| Corticosterone | 200 | 0.921 |
| Cortisol | 20000 | 0.006 |
| Danazol | 100000 | 0.001 |
| DHEA-S | 16000 | n. d. |
| Norgestrel | 1000 | 0.011 |
| Estradiol | 400 | n. d. |
| Ethisterone | 1000 | 0.001 |
| Ethynodiol diacetate | 1000 | n. d. |
| Medroxyprogesterone | 5000 | 0.004 |
| Norethindrone | 1000 | 0.004 |
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| Substance                     | Additive concentration ng/mL | Cross-reactivity % |
|-------------------------------|------------------------------|--------------------|
| Norethindrone acetate         | 1000                         | 0.008              |
| Testosterone                  | 2000                         | 0.069              |
| 21-Deoxycorticisol            | 2000                         | 0.067              |
| 11-Deoxycorticosterone        | 600                          | 3.92               |
| 11-Deoxycorticosterone        | 6000                         | 0.015              |
| 5α-Dihydrotestosterone        | 20                           | n. d.              |
| 5β-Dihydroprogesterone        | 240                          | 0.366              |
| Pregnenolone                  | 16000                        | 0.410              |
| Pregnanolone                  | 2000                         | 0.145              |
| Medroxyprogesterone acetate   | 1000                         | 0.003              |
| 6α-Methylprednisolone         | 1000                         | 0.003              |
| 17α-Hydroxyprogrenolone       | 2000                         | 0.009              |
| 17α-Hydroxyprogesterone       | 2000                         | 0.066              |
| 20α-Hydroxy-4-pregnen-3-one   | 250                          | 0.086              |

c) n. d. = not detectable

References
1. Johnson MR, Carter G, Grint C, et al. Relationship between ovarian steroids, gonadotrophins and relaxin during the menstrual cycle. Acta Endocrinol 1993;129:121-125.
2. Laufer N, Navot D, Schenker JG. The pattern of luteal phase plasma progesterone and estradiol in fertile cycles. Am J Obstet Gynecol 1982;143:808-813.
3. Veldhuis JD, Christiansen E, Evans WS, et al. Physiological profiles of episodic progesterone release during the midluteal phase of the human menstrual cycle: analysis of circadian and ultradian rhythms, discrete pulse properties, and correlations with simultaneous luteinizing hormone release. J Clin Endocrinol Metab 1988;66(2):414-421.
4. Filicori M, Butler JP, Crowley WF Jr. Neuroendocrine regulation of the corpus luteum in the human. J Clin Invest 1984;73:1638-1647.
5. Guillaume J, Benjamin F, Sicuranza B, et al. Maternal serum levels of estradiol, progesterone and h-Choriongonadotropin in ectopic pregnancy and their correlation with endometrial histologic findings Surg Gynecol Obstet 1987;165:9-12.
6. Thienpoint L, Siekmann L, Lawson A, et al. Development, Validation and Certification by Isotope Dilution Gas Chromatography-Mass Spectrometry of Lyophilized Human Serum Reference Materials for Cortisol (CRM 192 and 193) and Progesterone (CRM 347 and 348). Clin Chem 1991;37(4):540-546.
7. 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 (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

| CONTENT | SYSTEM | REAGENT | CALIBRATOR |
|---------|--------|---------|------------|
| Contents of kit | Analyzers/Instruments on which reagents can be used | Reagent | Calibrator |