Instruction for Use

【Product Name】

Progesterone (Electrochemiluminescence Immunoassay)

【Order Information】

REF NO.	Package Size
697023	50T
697024	2×50T
697021	100T
697022	2×100T

【Intended Use】

Immunoassay for the in vitro quantitative determination of progesterone in human serum and plasma. The results can be used in the auxiliary diagnosis for premonitory abortion.

Progesterone (PROG) is a steroidal hormone with a molecular weight of 314.5 daltons, which is mainly formed in the corput luteum, and the placenta during pregnancy. Synthetic pathways of various steroid hormones are basically uniform, except that in different tissues, final products of which are different due to diversity in enzyme systems. Synthetic steroid hormones can start from acetate, first synthesize cholesterol, or directly ingested blood cholesterol as a raw material for synthesis, Cholesterol is converted to pregnenolone by the action of mitochondrial enzymes. Pregnenolone is a precursor to synthesize progesterone, androgen and estrogen, and it's a Δ_5-steroid, which forms progesterone by the action of $\Delta_{5\text{-}4}$ isomerase. The first metabolite of progesterone is 17-alpha hydroxyprogesterone[1,2]. The main metabolite in the liver is pregnanediol, which is usually a water-soluble sulfate or glucosiduronide secreted by the kidney. Progesterone is maily transported by binding to albumin, by binding to corticosteroid-binding globulin (CBG) or sex hormone-binding globulin (SHBG)[2,3].

The secretion of progesterone changes periodically, and during the follicular phase of the menstrual cycle, progesterone level is lower. A rise in the progesterone level is observed one day prior to ovulation. Rapidly Increased progesterone synthesis occurs during the luteal phase and reaches a maximum concentration from 4 to 7 days after ovulation.These levels are decrease to baseline levels after 4 to 6 days, including menstrual periods[4,5]. Clinical studies have shown that progesterone plays a role in promoting ovulation and maintaining normal function of the corpus luteum in non-pregnant women, progesterone brings about the conversion of the uterine mucosa into a tissue rich in glands (secretion phase), in order to prepare for the intrauterine implantation of the fertilized ovum. If the progesterone produced by the corpus luteum is insufficient, it may indicate luteal dysfunction (LPD), while luteal dysfunction is associated with infertility and early abortion[6,7].

During the first 10 weeks of pregnancy, serum progesterone levels are maintained at relative peak levels, and progesterone levels rose slowly after 10 weeks of gestation until full term. In clinical studies progesterone can be used as an indicator of early abortion and a monitoring indicator for progesterone supplementation during abortion treatment. A decrease in progesterone concentration in early pregnancy suggests luteal insufficiency or abnormal embryonic development or both.

【Test Principle】

Competitive principle. Total duration of assay: 18 minutes.

20 µL sample was incubated with biotinylated PROG antibody. The binding sites of the labeled antibody become occupied by the sample analyte (depending on its concentration). After addition of streptavidin-coated microparticles and PROG antigen labeled with ruthenium, the 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 system reagent. 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 RFID.

【Main Components】

The reagent pack consists of MB, RA, RB, calibrators and control materials (Optional). Different batch of reagent components should not be used interchangeably.

Components	Ingredients	Volume (50T)	Volume (2×50T)	Volume (100T)	Volume (2×100T)
Magnetic Beads (MB)	Streptavidin-coated microparticles, 0.75 mg/mL; 0.1 M PBS; 0.05% ProClin™ 300	1×3.0 mL	2×3.0 mL	1×5.0 mL	2×5.0 mL
Reagent B (RB)	Anti-PROG-Ab~biotin , 0.5 µg/mL; 25 mM PBS; 0.05% ProClin™ 300	1×5.5 mL	2×5.5 mL	1×9.0 mL	2×9.0 mL
Reagent A (RA)	PROG-BSA~Ru(bpy)$_3^{2+}$, 0.057 µg/mL; 25 mM PBS; 0.05% ProClin™ 300	1×5.0 mL	2×5.0 mL	1×8.0 mL	2×8.0 mL
Calibrators	99.95% Calf serum, 0.05% ProClin™ 300	High: 1×1.0 mL Low: 1×1.0 mL			
Control Materials (Optional)	99.95% Calf serum, 0.05% ProClin™ 300	High: 1×1.0 mL Low: 1×1.0 mL			

Traceability：This method can be traced back to Roche Elecsys PROG.

See the control material value sheet for the target values and ranges of control materials.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic)

Additional materials for Automated ECL Immunoassay Analyzer eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Storage and Shelf Life】

Store at 2ºC-8°C.

Do not freeze.

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

Stability of the reagents	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks
on the analyzers at 2ºC~15ºC	8 weeks

The calibrators and control materials are stable up to the stated expiration date.

Stability of the calibrators and control materials	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks

The expiration date is labeled on the box, rackpack and bottles.

Damaged, expired or contaminated reagents should be discarded.

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

Human serum and plasma added with heparin lithium, EDTA-3K and EDTA-2K anti-coagulants are recommended. Blood samples should be collected by standard operation of venous puncture; after complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not recommended. Samples would better to be tested in 2 hours after collection, otherwise, should be stored 2~8 ºC for no more than 5 days or for 6 months at -25~-15 ºC. Freezing and thawing cycle is permitted only once. The samples were kept at room temperature for 24 hours.

【Assay Procedure】

Testing procedures and precautions

Previous to operation, users should read the operational manual carefully to be familiar with system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up progesterone (PROG) test according to the operational manual.

Put the PROG rackpack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration,the reagent and main curve information should be imported into the analyzer via radio frequency identification (RFID) reagent card. The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.

Recalibration is recommended when:

(1) The reagents of different lot are used;

(2) The same lot of reagents were used on the analyzer beyond 28 days;

(3) Quality control misses the target.

Quality control

For quality control, use PROG control materials.

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.

Follow the applicable government regulations and local guidelines for quality control.

Please make sure that the correct values are used.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is nmol/L, ng/mL or μg/L:

nmol × 0.314 = ng/mL (or μg/L); ng/mL (or μg/L) × 3.18 = nmol/L; ng/mL × 1 = μg/L

Specimen dilution

Samples exceeding the measuring range would be manually diluted with PROG-negative serum.

The recommended dilution ratio is 1:10, the concentration of the diluted sample must be >2 ng/mL, and the diluted sample test results need to multiply dilution ratio.

【Biological Reference Interval】

According to serum test results of 969 healthy humans (men 120; non-pregnant women 484; pregnant women 365), the percentile 5 to 95 gives reference intervals as the following sheet.

Due to differences in the region, race, gender and age, laboratories are recommended to set up their own reference ranges.

Subject		PROG (ng/mL)		
		Number	Median	5th~95th percentile
Healthy men		120	0.702	0.130~1.326
Healthy non-pregnant women	Follicular phase	121	0.819	0.162~1.521
	Ovulation phase	120	1.392	0.823~2.881
	Luteal phase	120	12.63	1.203~24.85
	Postmenopause	123	0.363	0.103~0.754
Healthy pregnant women	1st trimester	124	25.66	9.743~42.47
	2nd trimester	120	46.44	16.42~80.24
	3rd trimester	121	114.1	56.31~214.5

【Result Interpretation】

For explaining the result, the patient's overall clinical manifestation should be referred to, including symptoms, medical history and other relevant data and information.

【Limitations】

Test results are used only for clinical reference and cannot be used as the basis for confirmation or rule-out of diseases alone.

When the sample containing bilirubin ≤ 54 mg/dL, triglyceride ≤ 720 mg/dL, biotin ≤ 30 ng/mL, or hemoglobin ≤1 g/dL, total protein ≤10 mg/mL, interference deviation to the test result lies within ±10%. The test result would not be interfered by rheumatoid factor (1200 IU/mL).

【Measuring Range】

The measuring range of the kit is 0.1 ~ 60 ng/mL. For samples with PROG content below the lower detection limit, the results are reported in < 0.1 ng/mL; If the PROG content is above the upper detection limit, then the sample should be diluted and re-tested. The results are reported after multiplying with the dilution factor.

【Analytical Performance】

Lower limit of measurement

The limit of detection is 0.1 ng/mL.

Accuracy

The relative deviation of measurement result lies within±10% when measure the national standard.

The relative deviation of the measurement result lies within±10% when measure the metrologically traceable accuracy control materials.

Linearity

The correlation coefficient (Pearson's r) is not less than 0.9900 in the interval of 0.1 ~ 60 ng/mL.

Within-run imprecision (repeatability)

The coefficient of variation (CV) ≤ 8%.

Between-lot imprecision

The coefficient of variation (CV) ≤ 10%.

Analytical specificity

Corticosterone and 17α-hydroxyprogesterone were added to the samples containing the target concentration of 20 ng/mL (relative deviation ±15%) progesterone, so that the final concentration of corticosterone and 17α-hydroxyprogesterone were 10 ng/mL and 10 ng/mL, respectively. The mean of each sample shall be within M (the mean) ± 2SD (standard deviation) of the target concentration.

Method comparison

A comparison of the Lifotronic PROG assay (y) with the Roche Elecsys PROG method (x) using clinical samples gave the following correlations:

Number of samples measured: 281

Linear regression:

Y=1.0770 x-1.1619

r = 0.995

The sample concentrations were between approx 0.11~ 53.89 ng/mL.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.

Do not use expired or contaminated kits for in vitro diagnostics.

When using the kit, it's necessary to comply with regulations in the laboratory.

All reagents and samples including specimen, calibrators and control materials, should avoid foaming before and during test.

Test results of the kit are for clinical reference only, and clinical evaluation of patients should be combined with their symptoms and signs, medical history, other laboratory examination results and treatment responses.

samples with reagents from different manufacturers may get different results, and the results from different kits should not compare directly, lest cause the wrong medical explanation; It's suggested that laboratorians should point out the characteristics of used reagent in the test report to the clinician. In serial monitoring, if the reagent type is changed, a continuous parallel test should be performed and comparison of the results to the former to determine new baseline value.

This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.

Any serious incident that would be 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.

The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours /spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant.

【Symbols Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date	IVD	In vitro diagnostic medical device
LOT	Batch code	Σ	Sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limit		This way up
EC REP	Authorized representative in the European Community	CE	Indicates this device is in compliance with Europe Directive.
REF	Catalogue number	P/N	Material code
	Warning		Date of manufacture
UDI	Unique device identifier		

【Reference】

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6. Goldstein D, Zuckerman H, Harpaz S, et al. Correlation between estradiol and progesterone in cycles with luteal phase deficiency. Fertil. Steril. 1982;37(3):348-54.
7. Mais V, Cetel NS, Muse KN, et al. Hormonal dynamics during luteal-follicular transition. J. Clin. Endocrinol. Metab. 1987;64:1109-14.

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