Instruction for use
Progesterone Kit (Microfluidic Fluorescent Immunoassay)

Product name
Progesterone Kit (Microfluidic Fluorescent Immunoassay)
Abbreviated name: LYOFIA Progesterone

Ref. No. --- Package size
LMTHPG25C --- 25 Tests, LMTHPG25 --- 25 Tests (N-QC)

Package size
100 Tests, 50 Tests, 25 Tests, 10 Tests, 5 Tests, 100 Tests (N-QC), 50 Tests (N-QC), 25 Tests (N-QC), 10 Tests (N-QC), 5 Tests (N-QC).

Intended use
This device is intended to be used for the *in vitro* quantitative determination of Progesterone in human whole blood, serum or plasma. And it is for professional use only, not for self-testing of untrained individuals, nor for near-patient testing.

Summary
Progesterone is a steroid hormone with a molecular weight of 314.5D. It is mainly formed in the cells of the corpus luteum and the placenta during pregnancy. The concentration of progesterone is closely related to the growth and degeneration of the corpus luteum. It is almost undetectable during the follicular phase of the menstrual cycle, while the day before ovulation, the concentration of progesterone increases. During the luteal phase, the synthesis of progesterone increases, while pregnediol, the major degradation product of progesterone, is excreted in the urine during the second half of the menstrual cycle. Progesterone is able to cause the uterine mucosa to transform into gland-rich tissue (secretory phase). Progesterone assays for reproductive diagnosis: detection of ovulation and estimation of the luteal phase. Current clinical methods for detecting Progesterone include chemiluminescence method, immunochromatography method, and etc.

Principle
This product adopts the microfluidic fluorescence immunoluminescence method. The luminescent material relies on the external light source to obtain energy, then it is excited to make luminescence. And the immunological principle used is competition method. In addition, the microstructure in the strip inside the test cassette can make the reaction system to be uniformly mixed inside the test cassette, thereby improving the accuracy and precision of the detection result.

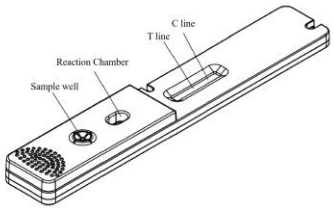

Figure 1: Schematic diagram of the test cassette

As shown in Figure 1, below the sample well is the lyophilized spheres placement tank. The lyophilized spheres are contained in the tank. The main component of the lyophilized sphere is the nanosphere (containing luminescent material) which is coupled with Progesterone monoclonal antibody I and DNP-BSA. The main component of T line is Prog-BSA, and the main component of C line is anti-DNP antibody.

The sample added from the sample well enters the flow microchannel through the microchannel valve and the microchannel mixer valve, so that the lyophilized spheres and the specimen in the lyophilized spheres placement tank are quickly dissolved and mixed evenly. The sample mixture flows along the microfluidic channel to the reaction chamber for reaction. The antigen in the specimen reacts with Progesterone monoclonal antibody I to form an antigen-antibody-nanosphere complex. The antigen-antibody-nanosphere complex will flow forward along the nitrocellulose membrane through the sample pad and can compete with the Prog-BSA immobilized on the T line of the nitrocellulose membrane. The more antigen in the sample, the less complexes will accumulate on the T line. The signal intensity is inversely proportional to the amount of captured antigen. In addition, the DNP-BSA in the reaction system can be captured by the anti-DNP antibody immobilized on the C line.

The fluorescence immunoassay analyzer used with the kit emits emission light, irradiates the T line and the C line, and excites the nanospheres to emit light, and then the specific signal values of the T line and the C line can be obtained.

The content of Progesterone in the sample can be determined using the calibration curve served in the Reagent information carrier.

Components and ingredients

<table>
<tr><th>No.</th><th colspan="4">Main components and ingredients</th></tr>
<tr><td rowspan="6">1</td><td rowspan="6">Test Cassette</td><td rowspan="3">Upper layer microfluidic chip</td><td rowspan="2">Fluorescent lyophilized spheres</td><td>Progesterone monoclonal antibody I</td></tr>
<tr><td>DNP-BSA</td></tr>
<tr><td>Blocking lyophilized spheres</td><td>Mouse IgG</td></tr>
<tr><td rowspan="3">Card shell (containing test strip)</td><td colspan="2">Sample pad</td></tr>
<tr><td rowspan="2">Nitrocellulose membrane</td><td>Prog-BSA</td></tr>
<tr><td>Anti-DNP antibody</td></tr>
<tr><td colspan="2">Absorbent paper</td></tr>
<tr><td colspan="3">PVC base plate</td></tr>
<tr><td>2</td><td>Sample Diluent</td><td colspan="3">Tris buffer</td></tr>
<tr><td></td><td></td><td colspan="3">Proclin300</td></tr>
<tr><td>3</td><td>Reagent information carrier</td><td colspan="3">A calibration curve is stored. The detection system is traceable to certified reference materials.</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">Control</td><td>Level 1</td><td colspan="2">Progesterone antigen</td></tr>
<tr><td>Level 2</td><td colspan="2">Progesterone antigen</td></tr>
</table>

Note:
- Sample Diluent (see the packaging label for the quantity);
- The kit whose packaging specifications describes "(N-QC)" do not contain quality control products;
- See the “target value list” for the target value range of the quality controls;
- The components from different lots of kits cannot be interchanged or mixed.

Storage and stability
Store the product at 2~30℃, it has a validity period of 18 months. Once the aluminum foil pouch of the test cassette is opened, the cassette has a validity period of 24 hours. After the control solution is reconstituted, seal and store it at 2~8℃ with a validity period of 4 hours. Do not use the test kit beyond the expiration date as indicated on label.

Applicable analyzer
Fluorescence immunoassay analyzer manufactured by Hunan Kangxin Biotechnology Co., Ltd., model LYOFIA-I, LYOFIA8.

Specimen requirements
1. This product is suitable for serum, plasma and whole blood samples. Lithium heparin, sodium heparin,EDTA and sodium citrate are the recommended anticoagulants for plasma and whole blood samples. The other anticoagulants have not been validated, they may affect the test results.
2. It is recommended that finishing the testing of whole blood, serum and plasma within 8 hours. If the specimens specified above cannot be used at once, store them at 2~8°C and finish the testing of whole blood within 24 hours or serum/plasma within 72 hours. Otherwise store the serum/plasma for up to 3 months at -20±5°C while the whole blood samples should not be frozen.
3. The samples to be tested should be free of precipitation. If precipitation occurs, centrifugation must be performed first. Do not use heat-inactivated samples.
4. Equilibrate the samples to ambient temperature before measurement. Cryopreserved samples should be completely thawed, rewarmed, and evenly mixed before use. Multiple

freeze-thaw cycles should be avoided. Do not use samples with significant hemolysis or blood clots.

Assay procedure

1. Assay preparation
1.1 Please follow this instruction for use and refer to the instruction manual of the fluorescence immunoassay analyzer.
1.2 Turn on the fluorescence immunoassay analyzer, check whether the analyzer can work normally, and prepare other related consumables.
1.3 Equilibrate the aluminum foil pouch to ambient temperature before opening.
1.4 Equilibrate the sample diluent and specimens to ambient temperature.
2. Calibration
Insert the reagent information carrier into the interface for the reagent information carrier on the analyzer LYOFIA-I or LYOFIA8, import the calibration curve stored in the reagent information carrier into the analyzer, and check whether the batch number of the reagent information carrier and the kit are consistent. Refer to the analyzer manual for specific operations.
3. Sample testing
3.1 Take out the test cassette has been equilibrated to ambient temperature and place it horizontally on a flat surface.
3.2 Dilute the sample with the sample diluent at the ratio of 1:3 (recommended procedure: pipette 65μL of sample into a centrifuge tube with 195μL of sample diluent). After mixing, take 65μL of the liquid and quickly add it into the sample well of the upper layer microfluidic chip (the small hole pointed by the arrow on the upper layer microfluidic chip). It is recommended to aspirate and dispense rapidly 3 times in the cassette hole.
3.3 Incubation and testing according to applicable instruments, as follows:
3.3.1 If the measuring instrument is LYOFIA-I, please insert the test card into the incubator immediately after adding the sample and then to let it stand for 10 minutes for reaction. Remove the test cassette after the end of the reaction, and insert it into the right position of the fluorescence immunoassay analyzer LYOFIA-I, click the “Test” for testing, and the analyzer will automatically scan the test cassette.
3.3.2 If the measuring instrument is LYOFIA8, please insert the test card into the test slot immediately after adding the sample, LYOFIA8 will automatically scan the test cassette, time the reaction and automatically detect after the reaction is over.
3.4 The fluorescence immunoassay analyzer automatically detects the results and calculates the content of Progesterone in the sample.
3.5 Take out the test cassette used and dispose it as medical waste.
4. Results Analysis
The measured fluorescence signal value can directly read the content of Progesterone in the sample from the calibration curve stored in the reagent information carrier of the corresponding batch. The default detection result is in nmol/L.
5. Quality Control
Each laboratory shall establish its own quality control system and rules according to relevant requirements.
To conduct quality control, you must use the quality controls of the same batch of the kit. The quality control product is lyophilized. After returning to ambient temperature, reconstitute it with purified water (show the target list for the water volume required), let it stand for at least 15 minutes, shake it horizontally and mix well, and then test the reconstituted control solution as a sample.

Reference interval

Male		≤ 4.45 nmol/L
Female	Follicular phase	≤ 4.77 nmol/L
	Ovulation period	2.54～9.54 nmol/L
	Luteal phase	5.41～85.86 nmol/L
	After menopause	≤ 2.54 nmol/L

It is recommended that each laboratory establish its own reference interval because Progesterone level determined is varied depending upon geographical, individual difference, or testing methods.

Result interpretation

The test results shall be only considered as a clinical reference rather than the unique basis for confirming or excluding a case. For diagnostic purposes, results should always be used in combination with clinical examination, medical history and other results of inspection.
nmol/L ×0.314=ng/mL
ng/mL ×3.18=nmol/L

Limitation

1. Possible causes of abnormal test results: Heterophilic antibodies, some non-specific components in blood with similar antigenic determinants can capture fluorescence-labeled antibodies.
2. Bacterial contamination of the sample or repeated freeze-thaw may affect the results.
3. Different brands and materials of blood collection tubes may affect the test results.
4. Samples with content close to or exceeding the upper limit of the linear range can be diluted with sample diluent, and the maximum dilution ratio is 1:1. The upper limit of the reportable range after dilution is 240 nmol/L.

Performance characteristics

1. Limit of detection: Not higher than 1 nmol/L.
2. Linearity: Linear interval is [1, 120] nmol/L; and the correlation coefficient |r| is not less than 0.9900.
3. Precision
3.1 Repeatability imprecision: The coefficient of variation (CV) is not more than 10%.
3.2 Within-laboratory imprecision: The coefficient of variation (CV) is not more than 10%.
3.3 Inter-lot imprecision: The coefficient of variation (CV) is not more than 15%.
4. Cross-reactivity
The potential interfering substances corticosterone and 17α-hydroxyprogesterone were respectively added into to the samples which contain progesterone. The interfering substance samples were made consist of progesterone around 64 nmol/L and 10 ng/mL of one of the interfering substances. The progesterone mean measurement value (2 replicates) of each interfering substance sample fallen within the M ± 2 SD (7 replicates) of the measurand values of a progesterone sample (64 nmol/L) in absence of the interfering substance.
5. Interference: Refer to the method of EP7-A2 "Interference Testing in Clinical Chemistry" to conduct the evaluation. If the relative deviation of the measurand value of the sample spiked with the interfering substance and that of the sample in absence of the interfering substance is not higher than 15%, the substance of no more than the corresponding study concentration may be considered no interference effect. Please see Table 1 for the upper limit of no interference of interfering substances to the assay:

Table 1: List of upper limits of no interference of interfering substances to the assay

Interference substance	Upper limits of no interference to the assay
Triglycerides	10 mg/mL
Bilirubin	0.3 mg/mL
Hemoglobin	6 mg/mL
Rheumatoid factor	50 IU/mL
Heterophilic antibodies	1: 10

Precautions and warnings

1. This product is an *in vitro* diagnostic reagent for single use and must not be reused.
2. The treatment, use, storage of the specimens and kits’ each component, and the disposal of solid and liquid wastes generated during the assay process should be handled in accordance with the corresponding measures of local biosafety guidelines or regulations.
3. Strictly follow operation procedure, and the correct result only be obtained under careful operation. Any modification to the operation procedure may affect the accuracy of the test results.

4. This product is sensitive to humidity, do not use if the foil pouch is damaged.
5. Do not insert the test cassette whose surface is wet with other liquids into the analyzer to avoid contamination and damage to the analyzer.
6. Keep away from vibration and electromagnetic environment when using the test cassette and fluorescence immunoassay analyzer.
7. Please see the outer label of the package of the kit for the production date and expiration date.

⚠This product contains chemical ingredients. Contacting with skin or mucosa should be avoided. If the product is spilled into eyes, mouth or skin accidentally, rinse with running water and seek for doctor advice if necessary.

⚠ This product contains animal-derived substances. Although it has passed the biosafety test, it does not rule out the risk of other potential infections. Please consider the kit and samples as potential sources of infection, and wear disposable gloves or take other measures to reduce the risk of infection during the detection process.

Symbols for use in the labeling

Symbols	Definition
	KEEP AWAY FROM SUNLIGHT
	TEMPERATURE LIMIT
IVD	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	CONSULT INSTRUCTIONS FOR USE
LOT	BATCH CODE
REF	CATALOG NUMBER
	USE-BY DATE
	DATE OF MANUFACTURE
	MANUFACTURER
Σ	SUFFICIENT FOR TESTS
	DO NOT RE-USE
	CAUTION
	KEEP DRY
	DO NOT USE IF PACKAGE IS DAMAGED
EC REP	AUTHORIZED REPRESENTATITVE IN THE EUROPEAN COMMUNITY

Bibliography

[1] Zhang Xuemei. Correlation between serum estradiol, progesterone and chorionic gonadotropin in early pregnancy and threatened abortion [J]. Electronic Journal of Practical Gynecology and Endocrinology, 2017,4(1):86,88.
[2] Yang Yanfei, Lan Cai'e. Analysis of serum progesterone, estradiol and human chorionic gonadotropin in early abnormal pregnancy [J]. Chinese Journal of Clinical Medicine for Women and Children (Electronic Edition), 2010,6(6):448- 450. DOI: 10.3877/cma.j.issn.1673-5250.2010.06.018.
[3] Mo Ping. Significance of α-HCG, estradiol and progesterone in the diagnosis and treatment of abnormal pregnancy[J]. Huaxia Medicine, 2000,13(4):417-418. DOI:10.3969/j.issn.1008 -2409.2000.04.002.

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Revision history

Version	Revision date	Change description
V01	2022-01-30	Initial