Instruction for use
Luteinizing Hormone Kit (Microfluidic Fluorescent Immunoassay)

Product name
Luteinizing Hormone Kit (Microfluidic Fluorescent Immunoassay)
Abbreviated name: LYOFIA LH

Ref. No. --- Package size
LMTHLH25C --- 25 Tests, LMTHLH25 --- 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 Luteinizing Hormone (LH) 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
In women's menstrual cycle, due to the regulation of the hypothalamic-pituitary-ovarian axis (HPOA), the follicles develop normally and ovulate. Gonadotrophin-releasing hormone (GmRH) secreted by the hypothalamus acts on the gonadotropin cells of the pituitary to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (luteinizing hormone, LH) in a pulsatile, and also acts on the ovary to promote the normal development and ovulation of follicles by the way of accepting the positive and negative feedback regulation of the ovary at the same time.
Both FSH and LH are glycoprotein hormones, which are formed by covalent bonding of two subunit peptide chains, α and β. They have the common α subunit structure, but the β subunit structure is different. The β subunit determines the specific antigenicity and specific function of the hormone, but it must be combined with the α subunit to form a complete molecule to have biological activity.
LH consists of an α-subunit composed of 92 amino acids and a β-subunit composed of 112-115 amino acids, encoded by a single gene on chromosomes 6 and 19. The LH receptor gene carries as many as 282 SNPs.
Like other hormones, LH has corresponding receptors and must bind to the corresponding receptors to exert biological effects. The LH receptor (luteinizing hormone receptor, LHR) gene is located on chromosome 2 and is mainly expressed in female theca cells (TCs). LH promotes androgen production in TCs, which in turn provides androstenedione as a substrate for estrogen synthesis. Androgens form estradiol through aromatization by granulosa cells. Polymorphic changes in the LH receptor may also lead to disease.
FSH and LH could work together to promote the growth and development of follicles and ovulation. Although FSH independently promotes follicle growth in the absence of LH, it may result in insufficient estradiol (E2) secretion and lack of capacity of luteinization and rupture. Initially, there is no LH receptor on granulosa cells. With the growth and development of follicles, FSH induces granulosa cells to generate LH receptors. Due to the upper limit of LH level varies among developmental stages and individual follicular development. When LH exceeds the upper limit, it inhibits the proliferation of granulosa cells, leading to follicular regression atresia or premature follicle luteinization, which suggests that LH has a negative selection effect on non-dominant follicles. During ovulation, the estrogen produced by the follicles has a positive feedback on the pituitary and hypothalamus to form the LH peak before ovulation, which can prompt the oocyte to resume the first meiosis and terminate in the middle of the second meiosis. The eggs are finally matured and released. During the luteal phase, the main role of LH is to maintain luteal function and promote the secretion of estrogen and progesterone.
The current clinical methods for detecting LH include chemiluminescence, immunochromatography and so on.

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 double antibody sandwich 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.

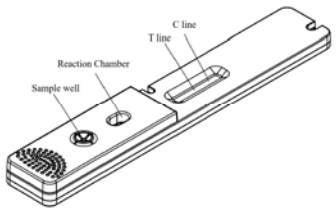

Figure 1: Schematic diagram of the test cassette

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 LH monoclonal antibody I and Chicken IgY antibody. The main component of T line is LH monoclonal antibody II, and the main component of C line is Goat anti-chicken IgY 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 LH 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 be captured by the LH monoclonal antibody II immobilized on the T line of the nitrocellulose membrane to form a double-antibody sandwich complex. In addition, the Chicken IgY antibody in the reaction system can be captured by the Goat anti-chicken IgY antibody immobilized on the C line. The more antigen in the sample, the more complexes will accumulate on the T line. The intensity of the fluorescent signal reflects the amount of captured antigen.
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 LH 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="8">1</td><td rowspan="8">Test Cassette</td><td rowspan="3">Upper layer microfluidic chip</td><td rowspan="3">Fluorescent lyophilized spheres</td><td>LH monoclonal antibody I</td></tr>
<tr><td>Chicken IgY antibody</td></tr>
<tr><td>Bovine serum albumin</td></tr>
<tr><td>Blocking lyophilized spheres</td><td>Mouse IgG</td></tr>
<tr><td rowspan="5">Card shell (containing test strip)</td><td colspan="2">Sample pad</td></tr>
<tr><td rowspan="2">Nitrocellulose membrane</td><td>LH monoclonal antibody II</td></tr>
<tr><td>Goat anti-chicken IgY antibody</td></tr>
<tr><td colspan="2">Absorbent paper</td></tr>
<tr><td colspan="2">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</td><td colspan="3">A calibration curve is stored. The detection system is</td></tr>
</table>

Instruction for use

Luteinizing Hormone Kit (Microfluidic Fluorescent Immunoassay)

	information carrier	traceable to certified reference materials.	
4	Control	Level 1	LH antigen
		Level 2	LH antigen

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 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 within 48 hours, or store them for up to 3 months at -20±5℃. Whole blood samples should be tested on the same day of collection and 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 65 μL of sample into a centrifuge tube with 195 μL of sample diluent). After mixing, take 65 μL 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 LH 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 LH in the sample from the calibration curve stored in the reagent information carrier of the corresponding batch. The default detection result is in mIU/mL.
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:
1.24-8.62 mIU/mL
Female:
Follicular phase: 2.12–10.89mIU/mL;
Ovulation phase: 19.18–103.03mIU/mL;
Luteal phase: 1.20–12.86mIU/mL;
Postmenopausal: 10.87–58.64mIU/mL;
It is recommended that each laboratory establish its own reference interval because LH level determined is varied depending upon geographical, individuals 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.

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 LH 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 500 mIU/mL.

Performance characteristics

1. Limit of detection: Not higher than 0.2 mIU/mL.
2. Linearity: Linear interval is [0.2, 250] mIU/mL; 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
4.1 Follicle stimulating hormone (FSH): Testing the sample whose concentration of FSH is not less than 200 IU/L, the measurement result using this kit is not higher than limit of detection.
4.2 Thyroid Stimulating Hormone (TSH): Testing the sample whose concentration of TSH is not less than 200 IU/L, the measurement result using this kit is not higher than limit of detection.
4.3 Human chorionic gonadotropin (hCG): Testing the sample whose concentration of hCG is not less than 1000 IU/L, the measurement result using this kit is not higher than limit of detection.
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 limit of no interference of interfering substances to the assay

Interference substance	Upper limit of no interference to the assay
Triglycerides	19 mg/mL
Bilirubin	0,66 mg/mL
Hemoglobin	0.01 mg/mL
Rheumatoid factor	1500 IU/mL

6. HOOK effect: LH samples with concentrations higher than 625 mIU/mL may have HOOK effect.

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

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Revision history

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