

Instruction for use

Testosterone Kit (Microfluidic Fluorescent Immunoassay)

Product name

Testosterone Kit (Microfluidic Fluorescent Immunoassay)
Abbreviated name: LYOFIA Testosterone

Ref. No. --- Package size

LMTHTE25C --- 25 Tests, LMTHTE25 --- 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 Testosterone 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

Testosterone is an anabolic steroid hormone in the human body and the main component of androgen. The production and secretion of testosterone is closely related to age, and is mainly produced by Leydig cells (LCs). Its secretion is regulated by luteinizing hormone (LH), and has a negative feedback effect to the pituitary-hypothalamic regulatory mechanism. Testosterone promotes the formation of male secondary sexual characteristics and maintains the function of the prostate and seminal vesicles. The vast majority of circulating testosterone is bound to carrier proteins (eg sex hormone binding globulin - SHBG). A woman's ovaries produce small amounts of testosterone. Physiological concentrations of androgens have no specific effect on women. Elevated androgen levels in women can lead to virilization (depending on the degree of increase in androgen levels). Measuring testosterone levels in women can help diagnose androgen syndrome (AGS), polycystic ovaries (Stein-Leventhal syndrome), and can also help suspicion of ovarian tumors, adrenal tumors, adrenal dysplasia, or ovarian dysfunction. Measuring testosterone levels in men can be used to diagnose conditions where testosterone production is insufficient, such as hypogonadism, estrogen therapy, chromosomal abnormalities (eg, Klinefelter syndrome), and liver cirrhosis. Current clinical methods for detecting Testosterone 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.

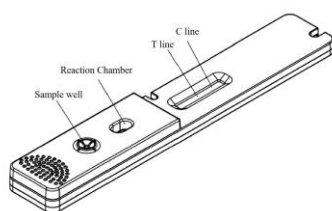


Figure 1: Schematic diagram of the test cassette

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 Testosterone monoclonal antibody I and DNP-BSA. The main component of T line is Tes-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 Testosterone 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 will not bind to the Tes-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 Testosterone in the sample can be determined using the calibration curve served in the Reagent information carrier.

Components and ingredients

Components and ingredients				
No.	Main components and ingredients			
1	Test Cassette	Upper layer microfluidic chip	Fluorescent lyophilized spheres	Testosterone monoclonal antibody I
				DNP-BSA
			Blocking lyophilized spheres	Mouse IgG
		Card shell (containing test strip)	Sample pad	
			Nitrocellulose membrane	Tes-BSA
				Anti-DNP antibody
	Absorbent paper			
	PVC base plate			
2	Sample Diluent	Tris buffer		
		Proclin300		
3	Reagent information carrier	A calibration curve is stored. The detection system is traceable to certified reference materials.		
4	Control	Level 1	Testosterone antigen	
		Level 2	Testosterone 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°C, 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°C with a validity period of 48 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 and sodium heparin 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

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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 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 Testosterone in the sample.

3.5 Take out the test cassette used and dispose it as medical waste.

Results Analysis

4. Results Analysis

The measured fluorescence signal value can directly read the content of Testosterone in the sample from the calibration curve stored in the reagent information carrier of the corresponding batch. The default detection result is in ng/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

Reference population	Group	Age (years old)	Reference interval (ng/mL)
Female	premenopausal	21-60	≤0.48
	postmenopausal	45-87	≤0.46
Male	<50	21-49	1.23~8.14
	≥50	50-89	0.9~7.80

It is recommended that each laboratory establish its own reference interval because Testosterone 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.

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 30 ng/mL.

Performance characteristics

1. Limit of detection: Not higher than 0.1 ng/mL.

2. Linearity: Linear interval is [0.1, 15] ng/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. 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 limit 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

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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.



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



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Revision history

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

Symbols for use in the labeling

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

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