Instruction for use
Total IgE Antibody Kit (Microfluidic Fluorescent Immunoassay)

Product name
Total IgE Antibody Kit (Microfluidic Fluorescent Immunoassay)
Abbreviated name: LYOFIA Total IgE

Ref. No. --- Package size
LMOTTE25C --- 25 Tests, LMOTTE25 --- 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 quantitative determination of total IgE 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.

Background
Immunoglobulin E (IgE) was discovered for its involvement in allergic reactions (Type I hypersensitivity).
Type I hypersensitivity is an allergic reaction provoked by re-exposure to a specific type of antigen referred to as an allergen. The sequence of events in the allergic reaction consists of the production of IgE antibodies in response to an allergen, binding of IgE to of mast cells, cross-linking of the bound IgE by the allergen upon re-exposure, and release of mast cell mediators such as histamine, lipid mediators and cytokines. Some mast cell mediators cause rapid increase in vascular permeability and smooth muscle contraction, resulting in many of the symptoms. The IgE concentration in serum is normally very low (<0.001% of the total serum immunoglobulin). The serum concentration of IgE is age-related, increasing during childhood until about 10 years of age, after which it reaches values that are maintained during adult life.
Measurement of total IgE is often used as a tool in the diagnosis and management of atopic diseases, and elevated level of IgE can be found in patients with allergic disease such as asthma, hay fever, atopic dermatitis and urticarial. It has been used to distinguish atopic from non-atopic individuals presenting allergy-like symptoms. In addition, studies have also shown that increased levels of IgE in cord blood and infants may be predictive of future atopic tendencies. Serum IgE levels may vary as a result of diet, genetic background, geographical location and other factors. It is therefore recommended that total IgE measurements be used in conjunction with other clinical tests when establishing diagnoses.

Principle
This product adopts the microfluidic time-resolved immunofluorescence assay. It is beneficial to eliminate the influence of fluorescent substances in the sample and the environment on the detection results by using time-resolved fluorescence for detection. 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.

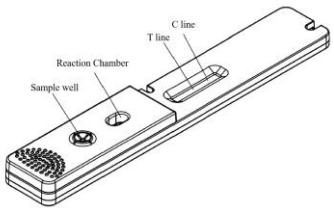

Figure 1: Schematic diagram of the test cassette

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 lanthanides) which is coupled with total IgE monoclonal antibody I. The main component of T line is total IgE monoclonal antibody II, and the main component of C line is Goat anti-mouse IgG 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 total IgE 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 total IgE monoclonal antibody II immobilized on the T line of the nitrocellulose membrane to form a double-antibody sandwich complex. In addition, the residual IgE monoclonal antibody I in the reaction system can be captured by the Goat anti-mouse IgG 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 fluorescence, and then the specific signal values of the T line and the C line can be obtained. The details of obtaining the signal is shown as the follows.
The analyzer takes advantage of the long-lived fluorescence of lanthanides and the fact that the Stokes shift (the wavelength difference between the maximum emission peak and the maximum absorption peak) of the emitted fluorescence is very large (mostly above 200 nm). The analyzer also depends on the difference in fluorescence lifetime of the fluorescent substances contained in the sample to introduce a certain delay time. After the short-lived background fluorescence is completely quenched, the long-lived specific fluorescence signal is measured.
After that, the content of total IgE 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="7">1</td><td rowspan="7">Test Cassette</td><td rowspan="2">Upper layer microfluidic chip</td><td rowspan="2">Fluorescent lyophilized spheres</td><td>IgE monoclonal antibody I</td></tr>
<tr><td>Bovine serum albumin</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>IgE monoclonal antibody II</td></tr>
<tr><td>Goat anti-mouse IgG 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
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 enterprise reference materials.</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">Control</td><td>Level 1</td><td colspan="2">IgE antigen</td></tr>
<tr><td>Level 2</td><td colspan="2">IgE 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. After the control solution is reconstituted, seal and store it at 2~8℃ with a validity period of 48 hours. Once the aluminum foil pouch

of the test cassette is opened, the cassette has a validity period of 24 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. Collect the blood sample after an overnight fast (approximately 10 hours).
2. 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.
3. 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 within 1 week, or store the serum and plasma for up to 1 month at -20±5°C.
4. The samples to be tested should be free of precipitation. If precipitation occurs, centrifugation must be performed first. Do not use heat-inactivated samples.
5. 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. The fully automatic analyzer has the functions of automatic sample addition and reagent addition. For the sample determination procedure of the fully automatic analyzer, please refer to the corresponding analyzer's manual. The following section 2.2 is an overview of the semi-automatic analyzer method.

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 and sample testing

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 1:100 (recommended procedure: pipette 10μL of sample into a centrifuge tube with 1000μL 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 Total IgE 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 total IgE in the sample from the calibration curve stored in the reagent information carrier of the corresponding batch. The default detection result is in IU/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

Age (years old)	95% Confidence interval（IU/mL）
＜1 year	＜15
1~5 year	＜60
6~9 year	＜90
10~15 year	＜200
＞15 year	＜100

It is recommended that each laboratory establish its own reference interval because total IgE 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. Samples with total IgE content close to or exceeding 1000 IU/mL can be diluted with sample diluent, and the maximum dilution ratio is 1:1. The upper limit of the reportable range after dilution is 2000 IU/mL.
3. Different brands and materials of blood collection tubes may affect the test results.

Performance characteristics

1. Limit of detection: Not higher than 1.0 IU/mL.
2. Linearity: Linear interval is [1.0, 1000] IU/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: Determine the IgA, IgM, IgG and IgD samples with concentrations no less than 100μg/mL respectively, and the measurement results shall all be no higher than 2 IU /mL.
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 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

6. HOOK effect: total IgE samples with concentrations higher than 1500 IU/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

Bibliography

[1] Reference values of total serum IgE and their significance in the diagnosis of allergy in young European adults. Int Arch Allergy Immunol 2007; 142: 230-238.

[2] Age-related serum immunoglobulin E levels in healthy subjects and in patients with allergic disease. J Allergy Clin Immunol. 1980; 66(4):305-313.

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Revision history

Version	Revision date	Change description
V01	2022-05-25	Initial