Instruction for use
Fecal Occult Blood Kit (Microfluidic Fluorescent Immunoassay)

Product name
Fecal Occult Blood Kit (Microfluidic Fluorescent Immunoassay)
Abbreviated name: LYOFIA FOB

Ref. No. --- Package size
LMTMFO25C --- 25 Tests, LMTMFO25 --- 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 qualitative detection of fecal occult blood (FOB) in human feces. And it is for professional use only, not for self-testing of untrained individuals, nor for near-patient testing.

Summary
Many diseases can cause hidden blood in the feces. This is also known as Fecal Occult Blood (FOB), Human Occult Blood, or Human Hemoglobin. In the early stages, gastrointestinal problems such as colon cancer, ulcers, polyps, colitis, diverticulitis, and fissures may not show any visible symptoms, only occult blood. Traditional guaiac-based methods lack sensitivity and specificity, and also have diet restrictions prior to testing.
The FOB Rapid Test Cassette (Feces) is a rapid test to qualitatively detect low levels of Fecal Occult Blood. Unlike guaiac assays, the accuracy of the test is not affected by the diet of the patients.
The current clinical methods for detecting FOB 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.

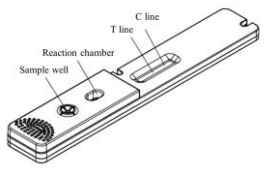

Figure 1: Schematic diagram of the test cassette

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 anti-hemoglobin antibody I and Chicken IgY antibody. The main component of T line is anti-hemoglobin 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 anti-hemoglobin 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 anti-hemoglobin 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 FOB 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="3">Upper layer microfluidic chip</td><td rowspan="2">Fluorescent lyophilized spheres</td><td>Anti-hemoglobin antibody I</td></tr>
<tr><td>Chicken IgY antibody</td></tr>
<tr><td>Blocking lyophilized spheres</td><td>Mouse IgG</td></tr>
<tr><td rowspan="4">Card shell (containing test strip)</td><td colspan="2">Sample pad</td></tr>
<tr><td rowspan="2">Nitrocellulose membrane</td><td>Anti-hemoglobin 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 rowspan="3">2</td><td rowspan="3">Sample collection tube</td><td colspan="3">Sampling rod</td></tr>
<tr><td colspan="3">Tris buffer</td></tr>
<tr><td colspan="3">Proclin300</td></tr>
<tr><td>3</td><td>Reagent information carrier</td><td colspan="3">A calibration curve is stored.</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">Control</td><td>Level 1</td><td colspan="2">Negative</td></tr>
<tr><td>Level 2</td><td colspan="2">Hemoglobin</td></tr>
</table>

Note:
- Disposable sample collection / treatment tube (including sample treatment solution, see the packaging label for the quantity)
- The kit whose packaging specifications describes "(N-QC)" do not contain quality control products;
- 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 4 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 feces samples.
2. The sample should be fresh, if it cannot be tested in time, store them at 2~8°C and finish the testing within 24 hours.
3. Equilibrate the samples to ambient temperature before measurement.

Sample collection
As shown in Figure 2, the stool sample collection and processing process involves the following steps:
1. Prepare the sampling tube, unscrew the lid and remove the sampling rod.
2. Insert the head of the sampling rod into the feces,and pick up the stool sample.
3. Place the sampling rod back into the tube, rotate tightly and shake to elute the sample. Pick samples from different locations and repeat the above sampling process three times. Test specimens

as soon as possible after sample collection for optimal test performance.

4. Before testing, please squeeze part of the sample (volume >200μL) from the collection tube into the new centrifuge tube before testing as pretreated sample.

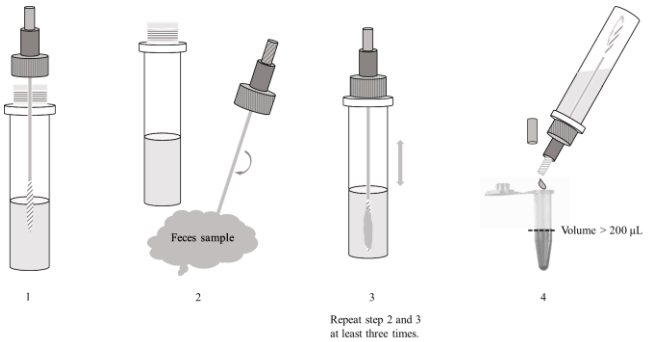

Figure 2: Sample collection and processing

Excessive/inadequate specimen collection or improper specimen handling/storage/transport may yield erroneous results.

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 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 or in an incubator.

3.2 Take 65 μL of the pretreated sample 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 FOB 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 FOB in the sample from the calibration curve stored in the reagent information carrier of the corresponding batch. Results are reported as the detection values for the corresponding sample.

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

If the test result is ≤ 1, the sample is judged as FOB negative;
If the test result is > 1, the sample is judged as FOB positive.

Limitation

1. The FOB Rapid Test Cassette (Feces) will only indicate the presence of Fecal Occult Blood, the presence of blood in feces does not necessarily indicate colorectal bleeding.

2. This product is only suitable for qualitative testing and cannot quantitatively detect the hemoglobin content in stool samples.

3. Possible causes of abnormal test results: menstrual samples or hematuria samples.

4. 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.

Performance characteristics

1. Precision

1.1 Repeatability imprecision: The coefficient of variation (CV) is not more than 10%.

1.2 Within-laboratory imprecision: The coefficient of variation (CV) is not more than 10%.

1.3 Inter-lot imprecision: The coefficient of variation (CV) is not more than 15%.

2. 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
Bovine hemoglobin	1 mg/mL
Chicken hemoglobin	1 mg/mL
Pork hemoglobin	1 mg/mL
Goat hemoglobin	1 mg/mL
Horse hemoglobin	1 mg/mL
Rabbit hemoglobin	1 mg/mL
Turkey hemoglobin	1 mg/mL

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 SUNLIFOBT
	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] Ostrow JD. Tests for Fecal Occult Blood. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths; 1990. Chapter 98.
[2] Simon JB. Occult Blood Screening for Colorectal Carcinoma: A Critical Review, Gastroenterology, 1985; 88: 820.

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Revision history

Version	Revision date	Change description
V01	2022-05-09	Initial