Instruction for use
PLGF & sFLT-1 Kit (Microfluidic Fluorescent Immunoassay)

Product name
PLGF & sFLT-1 Kit (Microfluidic Fluorescent Immunoassay)
Abbreviated name: LYOFIA PLGF & sFLT-1

Ref. No. --- Package size
LMPEPS25C --- 25 Tests, LMPEPS25 --- 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 placental growth factor (PLGF) and soluble fms-like tyrosine kinase 1 (sFLT-1) 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
PLGF is a member of the Vascular endothelial growth factor (VEGF) family, encoded by the PLGF gene, and is a glycosylated homodimeric protein involved in angiogenesis. The human PLGF gene is located in band 4, region 2 of chromosome 14, consisting of 7 exons, and 4 transcripts are formed by selective splicing of mRNA, PLGF-1, PLGF-2, PLGF-3 and PLGF-4. Among them, PLGF-2 and PLGF-4 have heparin-binding domains, encode cell membrane-associated proteins and function in an autocrine manner, while PLGF-1 and PLGF-3 function in a paracrine manner. Through autocrine and paracrine mechanisms, PLGF promotes trophoblast proliferation and differentiation, induces endothelial cell proliferation and migration, resists endothelial cell apoptosis, increases vascular permeability, and enhances the biological activity of VEGF[1], which is beneficial to the growth of the placenta, studies have shown that the expression of PLGF in normal non-pregnant women is very low and almost undetectable, while the expression level is increased in the placenta of normal pregnant women. The expression of PLGF is earlier during pregnancy, and it was detected in the serum of pregnant women at 8 weeks of gestation, but its expression was relatively low. From 10 weeks of gestation, the expression in serum increased successively with the increase of gestational weeks, peaked at 30 weeks of gestation, and then decreased due to the decline of placental function until delivery [2]. Placental growth factor is an important cytokine during pregnancy and plays an important role in promoting angiogenesis during the fetoplacental circulation. Research and development found that the decline in plasma PLGF levels in pregnant women occurred earlier than the onset of clinical symptoms of preeclampsia, and it is an excellent predictor of preeclampsia [1]. Vascular endothelial growth factor (VEGF) is a secreted glycosylated polypeptide factor. It is a pro-angiogenic factor that maintains normal endothelial function and promotes placental vascular development, while also maintaining the function of vascular homeostasis. Vascular endothelial growth factor has two receptors, VEGFR-1 and VEGFR-2, which promote and maintain the growth of vascular endothelial cells under the combined action of both. VEGFR-1 can be further divided into membrane VEGFR-1 (mVEGFR-1) and soluble VEGFR-1 (sVEGFR-1) according to the difference in structure. sVEGFR-1, also known as sFLT-1, is a polyglycosylated protein mainly produced and secreted in placental tissues. A type III receptor belonging to the tyrosine kinase family with tyrosine kinase activity. Studies have found that sFLT-1 has an anti-angiogenesis effect, can affect the permeability and integrity of vascular endothelial cells, and plays an important role in the construction of placental blood vessels [3]. In patients with preeclampsia, sFLT-1 can bind to VEGF-A and PLGF, respectively, and block the transduction of their pro-angiogenic signaling pathways, thereby causing systemic vasospasm and symptoms such as hypertension and proteinuria [3]. Early diagnosis of preeclampsia is very important to the health of pregnant women (parturients) and fetuses. Serological markers, especially angiogenesis factor markers represented by sFLT-1 and PLGF, show good auxiliary diagnostic performance, and have shown a positive role in the decision-making of hospitalization management of pregnant women with suspected preeclampsia[4].
The current clinical methods for detecting PLGF & sFLT-1 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.

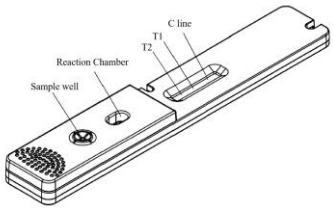

Figure 1: Schematic diagram of the test cassette

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 PLGF monoclonal antibody I and sFLT-1 monoclonal antibody I and DNP-BSA. The main component of T line is PLGF monoclonal antibody II and sFLT-1 monoclonal antibody II, 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 PLGF monoclonal antibody I and sFLT-1 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 PLGF monoclonal antibody II and sFLT-1 monoclonal antibody II immobilized on the T1 line，T2 line of the nitrocellulose membrane respectively to form a double-antibody sandwich complex. In addition, the DNP-BSA in the reaction system can be captured by the anti-DNP 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 PLGF and sFLT-1 in the sample can be determined using the calibration curve served in the Reagent information carrier.

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Components and ingredients

No.	Main components and ingredients			
1	Test Cassette	Upper layer microfluidic chip	Fluorescent lyophilized spheres	PLGF monoclonal antibody I
				sFLT-1 monoclonal antibody I
				DNP-BSA
			Blocking lyophilized spheres	Mouse IgG
		Card shell (containing test strip)	Sample pad	
			Nitrocellulose membrane	PLGF monoclonal antibody II
				sFLT-1 monoclonal antibody II
				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 enterprise reference materials.		
4	Control	Level 1	PLGF antigen and sFLT-1 antigen	
		Level 2	PLGF antigen and sFLT-1 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,LYOFIA-8.

Specimen requirements

1. This product is suitable for serum, plasma and whole blood samples. EDTA is 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 for whole blood within 24 hours or for serum and plasma for 48 hours, or store the specimens for up to 3 months at -20±5°C. 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 PLGF and sFLT-1 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 PLGF and sFLT-1 in the sample from the calibration curve stored in the reagent information carrier of the corresponding batch. The default detection result is in pg/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

Gestational week	sFLT-1 5th percentile (pg/mL)	sFLT-1 95th percentile (pg/mL)	PLGF 5th percentile (pg/mL)	PLGF 95th percentile (pg/mL)	sFLT/PLGF 5th percentile (pg/mL)	sFLT/PLGF 95th percentile (pg/mL)
10-14	652	2501	28.8	122	9.27	54.6
15-19	708	2807	66.2	289	3.51	25.7
20-23	572	2997	119	605	1.82	14.6
24-28	618	3205	169	1117	0.945	10.0
29-33	773	5165	114	1297	0.941	33.9
34-36	992	7363	78.0	984	1.23	66.4
37-Production	1533	9184	54.4	862	2.18	112

It is recommended that each laboratory establish its own reference interval because PLGF and sFLT-1 level determined is varied depending upon geographical, individuals difference, or testing methods.

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Auxiliary diagnosis

Early gestational phase (20+0-33+6 weeks)

	sFLT-1/PLGF ratio
Rule out cutoff	33
Rule in cutoff	85

Late gestational phase (34+0 weeks-delivery)

	sFLT-1/PLGF ratio
Rule out cutoff	33
Rule in cutoff	110

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. Samples with PLGF or sFLT-1 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.
4. Different brands and materials of blood collection tubes may affect the test results.

Performance characteristics

1. Limit of detection:

Analytes	LoD
PLGF	≤15 pg/mL
sFLT-1	≤100 pg/mL

2. Linearity:

Analytes	Linear interval	Correlation coefficient |r|
PLGF	[15, 10000]pg/mL	Not less than 0.9900
sFLT-1	[100, 85000]pg/mL	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. Analytical specificity: 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

5. HOOK effect: The concentration values that may introduce the HOOK effect are shown in the table below.

Analytes	Concentration
PLGF	25000 pg/mL
sFLT-1	200000 pg/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 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] Su Ming, Huang Jianhua, Zhang Ying. Clinical research p rogress of placental growth factor in predicting preeclampsia [J]. Medical Theory and Practice, 2021,34(17):2965-2967. DOI: 10.19381/j.issn.1001 -7585.2021.17.012.

[2] Yuan Kefen, Yang Xiaoyan. Research progress on the rela tionship between sFLT-1 and PLGF and early-onset preeclamp sia [J]. Inner Mongolia Medical Journal, 2019,51(10):1189-119 2. DOI:10.16096/J.cnki .nmgyxzz.2019.51.10.012.

[3] Gao Shang. Research progress of sFLT-1 related treatment in preeclampsia [J]. Journal of Practical Obstetrics and Gyne

cology, 2021,37(2):105-108.
[4] Gao Jinsong. Research progress of sFLT-1 level/PLGF level ratio in auxiliary diagnosis of preeclampsia [J]. Shanghai Medicine, 2018,39(1):19-21,59.

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Revision history

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