# Instruction for use

# C-Reactive Protein Kit (Microfluidic Fluorescent Immunoassay)

## Product name

C-Reactive Protein Kit (Microfluidic Fluorescent Immunoassay)
Abbreviated name: LYOFIA CRP

## Ref. No. --- Package size

LMFMCR25C --- 25 Tests, LMFMCR25 --- 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 C-Reactive Protein (CRP) in human whole blood, peripheral blood, serum or plasma. And it is for professional use only, not for self-testing of untrained individuals, nor for near-patient testing.

## Summary

C-reactive protein (CRP) is synthesized by hepatocytes. When the body is infected or tissue is damaged, macrophages and other leukocytes are activated to produce interleukin-6 (IL-6), interleukin-1 (IL-1), tumor necrosis factor TNF-a and other cytokines and other mediators, these cytokines and mediators reach the liver and stimulate hepatocytes and epithelial cells to synthesize C-reactive protein. Detection of C-reactive protein includes conventional C-reactive protein (conventional CRP) and high-sensitivity C-reactive protein (high-sensitivity CRP). The detection objects of the two are essentially the same, but the quantitative range of the detection methods is different. The concentration of conventional C-reactive protein can rapidly increase within a few hours of acute inflammation, and its increase is positively correlated with the degree of infection. It is a widely used as inflammatory marker and can be used to evaluate tissue damage and inflammatory diseases, and provide reference information for the diagnosis, treatment, and monitoring of disease. A common use of high-sensitivity C-reactive protein is as an aid in cardiovascular disease risk identification. Combined with the traditional clinical diagnosis of acute coronary syndrome, the high-sensitivity C-reactive protein result can be used as an early warning indicator of coronary artery disease or acute coronary syndrome recurrence. The detection range of full-range C-reactive protein (full-range CRP) covers the detection range of both conventional CRP and hypersensitive CRP.
Current clinical methods for detecting CRP 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 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.

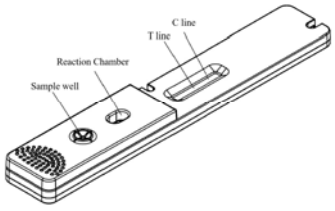


**Figure 1: Schematic diagram of the test cassette**

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 CRP monoclonal antibody I and chicken IgY antibody. The main component of T line is CRP 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 CRP 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 captured by with CRP monoclonal antibody II immobilized on the T line of the nitrocellulose membrane. 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 content of CRP 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="3">Fluorescent lyophilized spheres</td><td>CRP monoclonal antibody I</td></tr>
<tr><td>Chicken IgY antibody</td></tr>
<tr><td>Bovine serum albumin</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>CRP 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">PBS buffer<br>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 certified reference materials.</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">Control</td><td>Level 1</td><td colspan="2">CRP antigen</td></tr>
<tr><td>Level 2</td><td colspan="2">CRP 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. 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 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,whole blood and peripheral 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,

plasma and whole blood samples within 8 hours. If the specimens specified above cannot be used at once, store them at 2~8°C and finish the testing within 24 hours for whole blood sample, or within 72 hours for serum or plasma sample, or store serum samples for up to 3 months at -20±5℃. Whole blood and plasma samples should not be frozen. It is recommended to use fresh peripheral blood for testing.
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 1:500(Recommended procedure: pipette 3 μL of sample into a centrifuge tube with 1500 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 CRP 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 CRP in the sample from the calibration curve stored in the reagent information carrier of the corresponding batch. The default detection result is in mg/L.
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 sample diluent (show the target list for the sample diluent volume required), let it stand for at least 15 minutes, shake it horizontally and mix well. 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). Set the parameters according to the operational requirements of the manual of the applicable analyzer, let it incubate at 30± 2 ℃ for 5 minutes for reaction. After that, test on the machine and display/print the measurement results.

## Reference interval

High sensitive CRP: ≤1mg/L; General CRP: ≤10mg/L.
It is recommended that each laboratory establish its own reference interval because CRP 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. Samples with CRP 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 200 mg/L.
4. Different brands and materials of blood collection tubes may affect the test results.

## Performance characteristics

1. Limit of detection: Not higher than 0.4 mg/L.
2. Linearity: Linear interval is [0.4, 200] mg/L; 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. 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 10%, 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: CRP samples with concentrations higher than 500 mg/L 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] Li Shurui. The value of high-sensitivity C-reactive protein and interleukin-6 levels in the early diagnosis of acute exacerbation of chronic obstructive pulmonary disease [J]. Journal of Internal Medicine, 2019, 25(5): 401-403. DOI :10.11768/nkjwzzzz20190514.
[2] Zhang Suai, Song Chunhong, Chu Yan. The significance of serum high-sensitivity C-reactive protein procalcitonin and interleukin-6 in patients with acute cerebral infarction [J]. China Journal of Practical Neurological Diseases, 2019, 22(20): 2276-2280. DOI: 10.12083/SYSJ.2019.20.375.
[3] Liu Hongfei, Hou Wenquan. Observation on the therapeutic effect of C-reactive protein and interleukin-6 combined with routine blood test in patients with COPD [J]. Contemporary Medicine, 2019, 25(34): 167-168. DOI: 10.3969/j.issn .1009-4393.2019.34.071.

CE

Hunan Kangxin Biotechnology Co., Ltd.
Address: Room 301, 3rd Floor, Warehouse 5#, Block 6#, Xiangtan Comprehensive Free Trade Zone, 46#, Free Trade Road, Peace Street, Xiangtan Economic Development Zone, 411215, Xiangtan, PEOPLE'S REPUBLIC OF CHINA
Tel: +86 28 85155537
Website: www.vacurebiotech.com
E-mail: info@vacurebiotech.com

EC REP CMC Medical Devices & Drugs S.L.
Address: C/Horacio Lengo Nº 18, CP 29006, Málaga-Spain
Tel: +34 951 214054 Fax: +34 952 330100
E-mail: info@cmcmedicaldevices.com

## Revision history

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