Lifotronic

CE

Instruction for Use

【Product Name】

High-Sensitivity C-reactive Protein (Electrochemiluminescence Immunoassay)

【Order Information】

REF NO.	Package Size
689019	50T
689020	2×50T
689011	100T
689012	2×100T

【Intended Purpose】

Immunoassay for in vitro quantitative determination of C-reactive protein in human serum and plasma.

C-reactive protein (CRP) is composed of 5 identical non-glycosylated monomers, which are joined by non-covalent bonds to form disk-shaped pentamers with symmetrical structure. Each monomer contains 206 amino acid residues (23017 Da). The normal synthesis rate is 1~10 mg/d, so the C-reactive protein content in healthy people is very low. However, it can be synthesized in large quantities in the case of acute inflammation. More than 1 g can be synthesized every day and rapidly secreted into peripheral blood.

CRP is an important human reactive protein with no specificity in the acute phase. After 6~8 h and 24~48 h of infection, CRP reaches its peak, and the concentration in the acute phase could increase to thousands of times, and continuously increase with repeated inflammatory stimulation. Currently, it has been used as a routine hospital test item as auxiliary decisive basis in the diagnosis of many diseases. In addition, there's growing evidence that CRP is not only an inflammatory marker, but also directly involved in the inflammatory process. The clinical significance of CRP is the same as that of blood sedimentation, but not affected by factors such as red blood cells, Hb, lipid and age, and it appears earlier and disappears faster than blood sedimentation, which is, thus, a good indicator of inflammatory infection and curative effect[1,2].

Studies found that C-reactive protein is an independent risk factor for cardiovascular and cerebrovascular diseases[3, 4]. Some studies also further verified that hypersensitive C-reactive protein is not only a "risk factor" for cardiovascular and cerebrovascular diseases, but also a "monitoring marker" for treatment[5,6]. Hypersensitive C-reactive protein (HS-CRP) has been used in clinical applications of cardiovascular diseases in the United States. The risk of cardiovascular diseases can be stratified according to the CRP level: <1 mg/L was relatively low risk, 1.0~3.0 mg/L was moderate risk, >3.0 mg/L was high risk[7].

【Principle】

Sandwich principle.Total duration of assay：18 minutes.

The HS-CRP Kit adopts the double-site sandwich electrochemiluminescence method.The sample were diluted 25 times, biotinylated CRP monoclonal antibody and the ruthenium (Ru) complex-labeled CRP monoclonal antibody form an antigen- antibody sandwich complex, which is bound to streptavidin coated magnetic particles, and then transferred to the measuring cell where the magnetic particles are captured onto the surface of the electrode, while the unbound substances are washed away. Chemiluminescence is generated after the electrode is electrified, and the generated optical signal is measured by a photomultiplier, processed by an instrument, and the concentration of CRP in the sample is calculated based on the calibration curve.

【Main Components】

Components	Ingredients	Volume (2×50T)	Volume (50T)	Volume (100T)	Volume (2×100T)
(MB)	Streptavidin-coated microparticles 0.75 mg/mL::0.1 M PBS; 0.05%ProClin™ 300.	2×3.0 mL	1×3.0 mL	1×5.0 mL	2×5.0 mL
(RA)	Anti-CRP~Ru(bpy)$_3^{2+}$ 1.0 μg/mL:0.1 M PBS; 0.05% ProClin™ 300.	2×5.25 mL	1×5.25 mL	1×9.0 mL	2×9.0 mL
(RB)	Anti-CRP~biotin 1.0 μg/mL: 0.1 M PBS; 0.05% ProClin™ 300.	2×5.25 mL	1×5.25 mL	1×9.0 mL	2×9.0 mL
Diluent	0.1 M PBS, 0.05% ProClin™ 300.	2×7.5 mL	1×7.5 mL	1×13.5 mL	2×13.5 mL
Calibrator (High)	Calf serum, 0.05% ProClin™ 300	High: 2×1.0 mL Low: 2×1.0 mL	High: 1×1.0 mL Low: 1×1.0 mL		
Calibrator (Low)	Calf serum, 0.05% ProClin™ 300				
Control Material (High)	Calf serum, 0.05% ProClin™ 300	High: 2×1.0 mL Low: 2×1.0 mL	High: 1×1.0 mL Low: 1×1.0 mL		
Control Material (Low)	Calf serum, 0.05% ProClin™ 300				

ProClin™ is a trademark of the Dow Chemical Company or an affiliated company of Dow.

The assignment of calibrators value and this method can be traced back to WHO 1st IS 85/506.

See the Control Material RFID card for the target value and permissible range of Control Material.

【Instruments and Materials Required but Not Provided】

Additional materials for Automated ECL Immunoassay Analyzer eCL8000, eCL8000i, eCL8000p, eCL8000x:

- Auffer, 480 mL, 6×480 mL
- Buffer, 480 mL, 6×480 mL
- Concentrated Washing Buffer
- Assay Cup

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9000i, eCL9600, eCL9900, eCL9900i:

- Auffer, 2 L
- Buffer, 2 L
- Concentrated Washing Buffer
- Assay Cup, reaction cup
- Disposable Sampling Head, pipette tips
- PreClean, system wash solution
- Waste Bags, litter bags

【Applicable Instrument】

Automated ECL Immunoassay Analyzer: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL9000, eCL9000i, eCL9600, eCL9900, eCL9900i.

【Storage and Shelf Life】

The expiration date is labeled on the box, rackpack and bottles.Damaged, expired or contaminated reagents should be discarded.

The kit should be stored at

Stability	
Unopened at 2~8℃	12 months
Stored at 2~8℃	28 days
Stored onboard	28 days

【Specimen collection, handling and storage】

Human serum and plasma added with heparin lithium, heparin sodium, EDTA-K_2 and EDTA-K_3 anti-coagulants are recommended. Blood samples should be collected by standard operation of venous puncture; after complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not in suggestion. Samples would better to be tested in 6 hours after collection, otherwise, should be stored at 2~8℃ for no more than 12 hours or at -20℃ for no more than 1 month. Freezing and thawing cycle is permitted only once.

【Testing Procedure】

Testing procedures and precautions

Previous to operation, the system operation manual of the measuring instrument should be carefully read, so as to obtain relevant information such as system operating procedures, sample management, safety precautions and maintenance, and materials required for testing should be prepared. HS-CRP testing procedures should be set up in accordance with the system operating procedures.

Before using the reagent, put it into the analyzer 30 minutes in advance to automatically stir the magnetic bead particles and keep them in suspension.

Recommended environment temperature for testing: 10~30ºC relative humidity: ≤80.0%.

The HS-CRP test adopts the double-site sandwich electrochemiluminescence method, with the total test time of 18 minutes, and the test method is as follows:

1st incubation: The biotinylated monoclonal CRP-specific antibody and monoclonal CRP-specific antibody labeled with a ruthenium complex were added to the first reaction cup. In the second reaction cup, sample diluent and sample were diluted 25 times, and then the diluted sample was added to the first reaction cup and incubated together to form a sandwich complex.

2nd incubation: Then the system automatically aspirates 35 μL of streptavidin coated magnetic particles into the assay cup for incubation another 9 min. After addition of streptavidin-coated microparticles, the complex binds to the solid phase via interaction of biotin and streptavidin. After addition of streptavidin-coated microparticles, the complex binds to the solid phase via interaction of biotin and streptavidin.

After incubation, The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via calibration and a master curve provided via the reagent barcode.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification (RFID) reagent card (refer to instrument operational manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the

working curve automatically.
Recalibration is recommended when:
(1) the reagents of different lot are used;
(2) the same lot of reagents were used on the analyzer beyond 28 days;
(3) quality control misses the target.
(4)When changing the buffers of different lot numbers.

Quality Control procedure

In order to ensure the reliability of test results, it's recommended to test the control materials every 24 hours. After each calibration, reagent lot change, maintenance or failure repairment, quality control is recommended. The quality control results should fall within the scope of local regulations. If beyond, the analyzer status, reagents, calibration and other factors should be checked.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is ng/mL or mg/L: 1 mg/L = 1000 ng/mL.

【Biological reference interval】

According to 288 cases (male, 146; female, 142) of human serum samples in Guangdong hospitals, P. R. China, the percentile 2.5 to 97.5 draws a reference range for male and female was <748.9 ng/mL.
Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

【Limitations】

Test results are used only for clinical reference and cannot be used as the basis for diagnosis or rule-out of diseases alone.
The measuring range of the kit is 150~160000 ng/mL. Values below lower detection limit are reported as <150 ng/mL. Samples with CRP concentrations above the measuring range can be diluted with CRP sample diluent. The recommended dilution ratio is 1:20.
When samples contain the bilirubin concentration of 1 mg/mL or less, lipid concentration of 16 mg/mL or less, biotin concentration of 50 ng/mL or less, hemoglobin concentration of 6 mg/mL or less, Total proteins concentration of 30 mg/mL or less, HAMA concentration of 100 ng/mL or less, the interference bias of determination results deviation within ±10%. The kit is undisturbed by rheumatoid factor (800 IU/mL).

【Analytical Performance】

Detection limit

The detection limit is <150 ng/mL.

Accuracy

The international reference materials (NIBSC 85/506) is tested, and the relative deviation of its test results within ±15%.
The trueness control materials is tested, and the relative deviation of its test results within ±10%.

Linearity

The correlation coefficient (r) was not less than 0.9900 in the interval of 150~160000 ng/mL.
The absolute bias was no more than 1000 ng/mL in the interval of 150~10000 ng/mL.
The relative bias was no more than 15% in the interval of 10,000~160000 ng/mL.

Within-run precision (repeatability)

The coefficient of variation (CV) ≤ 8.0%.

Between-lot precision

The coefficient of variation (CV) ≤ 10%.

Analytical specificity

Test the following analog-additive blank samples, the results were lower than 150 ng/mL.

Analog	Concentration
HSA	2000 ng/mL

Homogeneity of calibrator and Control Materia

Within-bottle homogeneity: Coefficient of variation (CV) ≤ 8%,
Between-bottle homogeneity: Coefficient of variation (CV) ≤ 10%.

【Method Comparison】

A comparison of Lifotronic CRP assay with a commercially available CRP assay using clinical samples gave the following correlation:
y=1.0051x -52.525 correlation coefficient r=0.9970

【Precaution and Warning】

The kit is only used for vitro diagnostics.
When using the kit, it's necessary to comply with regulations in the laboratory.
All reagents and samples including specimen, calibrators and control materials, should avoid foaming before and during test.
Test results of the kit are for clinical reference only, and clinical evaluation of patients should be combined with their symptoms and signs, medical history, other laboratory examination results and treatment responses.
Due to factors such as methodology or antibody specificity, testing identical samples with reagents from different manufacturers may get different results, and the results from different kits should not compare directly, lest cause the wrong medical explanation; It's suggested that laboratorians should point out the characteristics of used reagent in the test report to the clinician. In serial monitoring, if the reagent type is changed, a continuous parallel test should be performed and comparison of the results to the former to determine new baseline value.
This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations. The preservative ProClin™ 300 contains 3% 2-methyl-4-isothiazolin-3-one (MIT) and 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT), will be harmful if inhalation, contact with skin and/or swallow, and may be toxic to aquatic organisms; thus, proper personal protection should be adopt while handling the reagent, and abandoned reagents should be dealed with in compliance with local regulations.

【Reference】

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