NEPHSTAR®
Blood C-Reactive Protein （bCRP）Kit

Catalog No. **BK051**

1. Intended Use
This product is used on NEPHSTAR® protein analysis system for quantitative determination of human C-Reactive Protein (CRP) in whole blood as an aid in diagnosis and treatment of inflammatory conditions, bacterial infection as well as cardiac diseases.

2. Summary
CRP is synthesized in liver and consists of five identical polypeptide chains forming a five-membered ring of molecular weight of 120 kD. CRP is one of the most characteristic acute-phase proteins and is considered as a reliable indicator of disease activity in various clinical conditions. Its concentration in blood increases rapidly by
as much as 1000-fold upon exposure to various inflammatory stimuli. CRP has been used successfully for clinical diagnosis and monitoring of a variety of infections and diseases, including infections caused by bacteria, fungi, and viruses ; intercurrent infections in leukemia and systemic lupus erythematosus; noninfectious inflammatory diseases such as rheumatoid arthritis; and diseases with cellular necrosis such as myocardial infarction. Measurements of CRP are especially useful in distinguishing viral from bacterial infections.
Traditional method of measuring CRP concentration is using serum as sample, which means more steps and more time to attain the goal. Goldsite therefore develops this Blood CRP kit using whole blood as testing sample. With this kit CRP concentration can be measured with venous blood or capillary blood and the procedure is simpler and quicker.

3. Test Principle
Particle-enhanced immunonephelometry is applied. This method involves measuring the light scattered by insoluble complexes formed by reaction between specific protein in samples and its respective antibody covalently coupled to latex particles, and the amount of scattered light is directly proportional to the concentration of the protein under condition that antiserum is in excess. The latex particles increase the size of complexes formed and thus the amount of light as well as the test sensitivity. Concentrations are automatically calculated by reference to a calibration curve stored in the instrument.

4. Kit Components

Code	Name	Volume/Quantity
BA051	bCRP Antiserum	1×2.0 ml
BY051	bCRP Sample treatment solution	1×55.0 mL
BB051	bCRP Reaction buffer	1×25.0 mL
BC051	bCRP Magnetic card	1
BM051	bCRP Control	1×0.3mL
	Manual	1

5. Materials required but not supplied
5.1 NEPHSTAR Protein analysis system（NS100）
5.2 NEPHSTAR Accessory pack（DK110）
5.3 Electronic pipette（YB201）
5.4 Pipette 5-50uL (YB301)
5.5 Pipette 100-1000uL(YB302)
5.6 Equipment for collection of Samples

6. Storage and Stability
The unopened reagent kit should be stored under 2-8℃ and can be used until the expiry date labeled on the kit. Do not freeze! The buffer should be equilibrated to room temperature before use. Once opened store the antisera and control at 2-8℃ and the buffer at **18-25℃ and be sure to screw on the cap tightly.** Under these conditions the buffer is stable for 3 months, antisera and control for 1 month.

7. Sample Collection And Preparation
Use whole blood samples. Capillary blood or venous blood can be used. If the samples are not anti –coagulated, the samples should be mixed with the bCRP sample treatment solution as soon as they are collected. If stored at 2-8℃ the anti-coagulated samples are stable for as long as 72 hours. If stored under -20℃ they are stable for several months. Samples should be treated on the day of assay. Testing of the following types of sera may result in misleading values:
7.1 Highly lipemic, turbid samples are not suitable for nephelometric assays and should not be used unless centrifuged or prepared using other methods. If the background is too turbid and can not be removed, please think of other measuring method.
7.2 Testing of samples containing rheumatoid factors, paraproteins or circulating immunocomplexes can result in misleading values due to non-specific scattering light possibly generated by these articles.

8. Test Procedure
Summary: Reagent volumes added to the cuvette

Reagent	Volume
Sample（1/41）	40ul
bCRP Reaction Buffer	400ul
bCRP Antiserum	40ul

8.1 Switch NEPHSTAR on.
8.2 Enter chemistry number. Enter chemistry number of bCRP kit (bCRP=51). If bCRP assay has never been performed on the instrument before, please swipe card when “please swipe card” is displayed.
8.3 The assay name and lot of reagent are displayed. Check carefully, press ENTER if the lot number is identical to that printed on the card or kit label, otherwise swipe card to update the curve data stored in NEPHSTAR.
8.4 Treat samples or controls. The treatment scheme for bCRP assay is 1/41. Add 20uL whole blood and 800uL treatment solution to a tube. Mix one minute or more until the mixture are transparent without any visible particle.
8.5 Prepare one cuvette for each sample to be assayed. Place a stirring bar to the cuvette using the forceps supplied with NEPHSTAR, then add 40uL of treated sample carefully to the bottom of the cuvette.
8.6 Enter sample ID. Press number keys to enter the sample ID; or press ENTER to accept the currently displayed sample ID.
8.7 Enter sample dilution: 41. This sample dilution (treatment) scheme should not be changed. Press enter to accept.

8.8 Place cuvette in chamber. Place the cuvette containing a stirring bar and 40uL of treated sample in the chamber and press it down slightly until it reaches the bottom of the chamber. The cuvette will be detected automatically.

8.9 Add reagent. Add 400 uL bCRP reaction buffer and 40 uL bCRP antiserum simultaneously into the cuvette using the electronic pipette （Cat. No.: YB201）supplied with NEPHSTAR. NEPHSTAR will sense the addition of reagents. With movement of the stirring bar, the assay begins after blanking and result will be printed automatically at the end of the assay.

8.10 On completion of the assay remove the cuvette, press ENTER to perform the next assay. Sample ID will increase sequentially. For alteration of the ID press BACK twice and tip in the right number.

8.11 If NEPHSTAR indicates result is higher or lower than measurement range, reassay the sample with other method such as NEPHSTAR CRP kit (DK022).

8.12 On completion of all assays of the same chemistry press ESC and return to step 8.2. Enter new chemistry number and begin another assay.

9. Quality Control

In accord with good laboratory practice, users should run control with every batch of samples. Results of control should fall in the validity range labeled on the control vial.

10. Adjustment of result

The result is calculated on presumption that the hematocrit (HCT) is 40. If the exact HCT of the tested sample is known, the result can be adjusted according to the formula below:

$$\frac{60}{100\text{-HCT}} \times \text{CRP result}$$

11. Sensitivity and measuring range

The sensitivity limit is 1.0mg/L and the upper limit is 350mg/L when the default dilution scheme is applied.

12. Antigen Excess

Sample concentration of less than 6000mg/L will not result in antigen excess, when the results will be misleadingly low. But this situation will almost impossible to happen.

13. Reference Range

12.1 According to CRM470，normal range of CRP concentration of healthy adult is：<5mg/L. We recommend local reference ranges are produced.

12.2 Diagnosis and treatment can not only depend on determination of bCRP. The clinical symptoms and other laboratory findings of respective patients should be taken into consideration。

14. Precision

Two analyte concentrations are assayed within several days using this kit of the same lot on NEPHSTAR. 20 repeat assays are performed for each concentration. The average coefficient variations (CV) for each concentration are displayed in the following table:

CRP（mg/L）	CV（%）
17.5	2.36
128.6	3.02

15. Correlation Study

A correlation study is performed on 20 clinical blood samples using this kit on NEPHSTAR and serum samples prepared with the respective blood samples using Behring CRP reagent on BNII. The linear regression equation and correlation equation got as showed below demonstrate a good correlation between the two methods:

Y=0.958X – 0.152

(Y= NEPHSTAR® bCRP，X=BNII™ CRP)

Correlation coefficient r=0.979

16. Caution And Warning

16.1 The reagents are only for in vitro diagnostic use。

16.2 The reagents can be used only by trained personnel and good laboratory practice (GLP) and the stated procedure should be abided strictly.

16.3 All sera have been tested to be HIV(1&2) antibody negative, HBsAg negative. However, the performed testing method can not assure the absolute absence of infectious agents in blood products, so please be sure to handle the blood products such as controls and antisera as potentially infectious sources.

16.4 All reagents of the kit contain sodium azide as preservative. Take caution when handling them. Ingest or contact of the reagents with skin or mucous membranes is forbidden. Wash with large amount of water and seek medical advice if contact does occur. In addition, explosive metal azides may be formed with lead or copper plumbings; when disposing the reagents be sure to flush with large amount of water to avoid buildup of azide.

16.5 All components of kit are NEPHSTAR® specific. **Reagents of different lots are not interchangeable**, otherwise the results can be misleading.

17. Referrences

17.1 Urdal P, Borch SM, Landaas S, Krutnes MB, Gogstad GO, Hjortdahl P. Rapid immunometric measurement of C-reactive protein in whole blood. Clin Chem 1992;38:580-584.

17.2 Chambers RE, Whicker JT, Dieppe PA. Acute phase proteins in inflammatory disease. Clin Diagnosis Lab 1988;1:29-37.

17.3 Morley JJ, Kushner I. Serum C-reactive protein levels in disease. Ann N Y Acad Sci 1982;389:406-18.

17.4 Lindback S, Heligren U, Julander I, Hanseon LO. The value of C-reactive protein as a marker of bacterial infection in patients with septicaemia, endocarditis and influenza. Scand J Infect Dis 1989;21:543-.9.

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