# NEPHSTAR®
# Ultrasensitive C-Reactive Protein （UsCRP）Kit

**Catalog No.** **DK025**

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

## 2. Summary
C-Reactive Protein (CRP) is an acute marker of inflammatory processes. In case of an acute inflammation the concentration of CRP increases and decreases more quickly than the red cell sedimentation rate. The increase of CRP occurs in a non-specific way in different kinds of tissular aggression, as for example in infectious states, rheumatoid arthritis, myocardium infarct, malignant tumour, etc.
Routinely available immunochemical assay methods for CRP have limited sensitivity, and until recently, CRP concentrations below 10 mg/L could not be measured precisely, leading to the wide spread adoption of this value as the upper limit of the health-associated reference range. This is satisfactory for most purposes in general medicine.
However, in neonatal pediatric practice, a high sensitive CRP immunoassay shows that health-associated reference values are below 1 – 2 mg/L and that any rise above such values is associated with serious disease, usually bacterial infection.
More recently, application of sensitive CRP assays to studies of adult cardiovascular disease has revealed important prognostic relationships between modest increase of CRP and the occurrence, progression, and thrombo-occlusive complications of atherosclerosis. We therefore developed an ultra sensitive CRP assay with a detection limit around 1.0 mg/L and a high measuring range (0 – 150 mg/L CRP).

## 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 |
|---|---|---|
| DA025 | UsCRP Antiserum | 1×2.0 ml |
| DB025 | UsCRP Reaction buffer | 1×25.0 mL |
| DC025 | UsCRP Magnetic card | 1 |
| DM025 | UsCRP Control | 1×0.5mL |
| | 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 serum samples. Collect blood samples by venepuncture and let them clot naturally and separate the sera as soon as possible to prevent haemolysis. Sera may be stored at 2-8°C for 48 hours, otherwise freeze at –20°C or below; do not freeze and thaw sera more than once. Sample dilutions should be freshly prepared on the day of assay. Testing of the following types of sera may result in misleading values:
7.1 Highly lipemic, turbid and haemolysed 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（1/5） | 40ul |
| UsCRP Reaction Buffer | 400ul |
| UsCRP Antiserum | 40ul |

8.1 Switch NEPHSTAR on.
8.2 Enter chemistry number. Enter chemistry number of UsCRP kit (UsCRP=25). If UsCRP 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 Dilute samples or controls using NEPHSTAR Sample Diluent supplied in NEPHSTAR Accessory pack (Cat. No: DK110）. The default dilution scheme for UsCRP assay is 1/5 (e.g. 160uL sample diluent + 40uL sample）。
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 diluted 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: 5. Accept the default sample dilution by pressing ENTER, otherwise press number keys to alter the dilution scheme.

8.8 Place cuvette in chamber. Place the cuvette containing a stirring bar and 40uL of diluted 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 UsCRP reaction buffer and 40 uL UsCRP 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 than measurement range, reassay the sample at a higher dilution of e.g. 1/55 （400μL sample diluent + 40μL 1/5 diluted sample）. Accordingly the sample dilution should be altered to 1/55 （press BACK and then the number keys to alter the sample dilution）。

8.12 If NEPHSTAR indicates result is lower than measurement range, reassay the sample at a lower dilution of e.g. 1/1 （undiluted）. Accordingly the sample dilution should be altered to 1 (press BACK and then the number keys to alter the sample dilution）。

8.13 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. Sensitivity and measuring range

The sensitivity limit is 0.25 mg/L and the upper limit is 18mg/L when the default dilution scheme is applied. The sensitivity limit is 0.05mg/L when samples are assayed undiluted.

## 11. Antigen Excess

Sample concentration of less than 750mg/L will not result in antigen excess, when the results will be misleadingly low. On suspicion of antigen excess please reassay the sample at dilution of 1/200 (780uL sample diluent + 20uL 1/5 diluted sample).

## 12. Reference Range

12.1 According to literature，normal range of UsCRP concentration of healthy adult is：<3mg/L. We recommend local reference ranges are produced.

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

## 13. 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:

| UsCRP （mg/L） | CV （%） |
|---|---|
| 1.8 | 2.68 |
| 12.6 | 3.12 |

## 14. Correlation Study

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

Y=0.971X-0.09

(Y= NEPHSTAR® UsCRP，X=BNII CRP)

Correlation coefficient r=0.985

## 15. Caution And Warning

15.1 The reagents are only for in vitro diagnostic use。

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

15.3 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.

15.4 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.

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

## 16. Referrences

1. Claus DR, Osmand AP, Gewurz H. Radioimmunoassay of human C-reactive protein and levels in normal sera. J Lab Clin Med 1976;
87: 120-128

2. Wasunna A, Whitelaw A, Gallimore R, Hawkins PN, Pepys MB. C-reactive protein and bacterial infection in preterm infants. Eur J Pediatr 1990; 149:424-427

3. Heinrich J, Schulte H, Schönfeld R, Köhler E, Assmann G. Association of variables of coagulation, fibrinolysis and acute-phase with atherosclerosis in coronary and peripheral arteries and those arteries supplying the brain. Thromb Haemostas 1995; 73: 374-379

**Goldsite Diagnostics Inc.**
#3A & 4A, Technology Building Annex
Nanhai Road, Nanshan district
Shenzhen, P.R.China, 518067
Tel: 86 755 26890807

Fax：86 755 26890799

EC REP

**CMC MEDICAL DEVICES & DRUGS, S.L.**
C/ Horacio Lengo No 18, CP 29006, Málaga-Spain

IVD CE