NEPHSTAR®
Rheumatoid Factor (RF) Kit

Code No. DK023

1. Intended Use
This product is used on NEPHSTAR® protein analysis system for quantitative determination of human rheumatoid factor (RF) in serum as an aid in diagnosis of rheumatoid arthritis.

2. Summary
Rheumatoid factors are a heterogeneous group of autoantibodies directed against the antigenic determinants on the Fc-region of IgG molecules. They are important in the diagnosis of rheumatoid arthritis, but can also be found in other inflammatory-rheumatic diseases and in various non-rheumatic diseases. They are also found in clinically healthy persons over 60 years of age. Despite these restrictions, the detection of rheumatoid factors is a diagnostic criterion of the American College of Rheumatology for classifying rheumatoid arthritis. These autoantibodies occur in all the immunoglobulin classes, although the usual analytical methods are limited to the detection of rheumatoid factors of the IgM type.

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 antibody 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
DA023	RF Antiserum	2×0.45 ml
DB023	RF Reaction buffer	1×25.0 mL
DC023	RF Magnetic card	1
DM023	RF 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 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（1/20）	40ul
RF Reaction Buffer	400ul
RF Antiserum	15ul

8.1 Switch NEPHSTAR on.

8.2 Enter chemistry number. Enter chemistry number of RF kit (RF=23). If RF 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 RF assay is 1/20 (e.g. 380uL sample diluent + 20uL 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: 20. 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 RF reaction buffer and 15 uL RF 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. 1/220 (200μL sample diluent + 20μL 1/20 diluted sample). Accordingly the sample dilution should be altered to 1/220 (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. 1/11 (200μL sample diluent + 20μL sample). Accordingly the sample dilution should be altered to 1/11 (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 20 IU/mL and the upper limit is 300 IU/mL when the default dilution scheme is applied. The sensitivity limit is 11 IU/mL when samples are diluted at 1/11.

11. Antigen Excess

Sample concentration of less than 8000 IU/mL will not result in antigen excess. But such high RF concentration of patient sample will not happen.

12. Reference Range

12.1 According to WHO, normal range of RF concentration of healthy adult is: <30 IU/mL. We recommend local reference ranges are produced.

12.2 Diagnosis and treatment can not only depend on determination of RF. 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:

RF (IU/mL)	CV (%)
50	1.89
324	2.02

14. Correlation Study

A correlation study is performed on 20 clinical serum samples using this kit on NEPHSTAR and Beckman Array RF reagent on Array 360. The linear regression equation and correlation equation got as showed below demonstrate a good correlation between the two methods:

Y=0.982X - 3.32

(Y= NEPHSTAR® RF, X=Array RF)

Correlation coefficient r=0.972

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

16.1 Arnett FC, Edworthy SM, Bloch DA et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-324

16.2 Bartfied H. Distribution of rheumatoid factor activity in nonrheumatoid states. Ann NY Acad Sci 1969;168:30-40

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