Nephstar
C4 Kit
For determination of Complement 4

Code	DK034

1. Intended Use
This product is used on Nephstar Specific Protein Analyzer for quantitative determination of Complement 4 (C4) in human serum or plasma as an aid in the diagnosis of abnormal C4 metabolism.

2. Summary
The complement system can be activated via the classical and the alternative pathways. C4 participates in activation in the classical pathway. A decrease in C4 is common, but complete absence is rare. A lowered concentration or the complete absence of C4 occurs in immunocomplex diseases, Systemic Lupus Erythematosus (SLE), autoimmune thyroiditis and juvenile dermatomyositis. The commencement of SLE in patients with C4 deficiencies can often be detected at a very early stage, and the course of the disease is milder than in patients with normal complement levels. Infections such as bacterial and viral meningitis, streptococcal and staphylococcal sepsis and pneumonia are reported to be associated with decreased C4.
Additional differentiation can be obtained by the determination of C4 when the level of C3 is low. If in such cases the concentration of C4 is normal, then an activation of the alternative pathway is likely. The main use of C4 determinations is in assessing the course of hypocomplement conditions. As an acute phase protein, C4 is produced to an increased extent during inflammatory processes. It is elevated in systemic infections, noninfectious chronic inflammatory conditions (primarily chronic polyarthritis) and physiological states (pregnancy). The elevation rarely exceeds twice the normal value and can mask a reduction in the current consumption.

3. Test Principle
Immunonephelometry is applied. This method measures the light scattered by the insoluble immune complexes formed between C4 in the sample and its specific antibodies, and the amount of light scattered is directly proportional to the concentration of C4 when the antiserum is in excess. The C4 concentration is automatically calculated by reference to a calibration curve stored in the instrument.

4. Kit Components

Nephstar C4 Kit				
Cat No.	**Package**	**Component Code**	**Component Name**	**Component Volume/Quantity**
NS34050	50T	DA034	C4 Antiserum	1 × 2.0 mL
		DB034	C4 Reaction Buffer	1 × 25.0 mL
		DC034	C4 Magnetic Card	1
		DM034	C4 Control	1 × 0.3 mL
			Manual	1

5. Materials Required but Not Supplied
5.1 Nephstar Specific Protein Analyzer (NS100)
5.2 Nephstar Accessory Pack (NSAS200), the pack contains cuvettes, stirrers, and the Sample Diluent
5.3 Pipette 5 - 50 µL, 100 - 1000 µL
5.4 Equipment for collection of samples

6. Storage and Stability
Store the C4 Antiserum and the C4 Control at 2 - 8°C. The unopened antiserum and control are stable until the expiry date labeled on the vials. Once opened, they are stable for 30 days. Store the C4 Reaction Buffer at 2 - 30°C. The unopened buffer is stable until the expiry date labeled on the vial. Once opened, it is stable for 30 days.

7. Sample Collection and Preparation
Fresh serum and plasma samples can be used. Samples can be kept at 2 to 8°C for up to 72 hours. Samples can be kept longer at -20°C or below. Do not freeze and thaw samples more than once. Testing of the following types of samples may result in misleading values:
7.1 Highly lipemic, turbid and haemolyzed samples are not suitable for nephelometric assays and should not be used unless centrifuged or prepared in advance. If the background is too turbid to be cleared, please consider alternative measuring methods.
7.2 Testing of samples containing rheumatoid factors, paraproteins or circulating immunocomplexes may result in misleading values due to non-specific scattering light generated by these articles.

8. Test Procedure
Summary: Reagents added to the cuvette

Reagents	Volume
Diluted sample (1/11)	40 µL
C4 Reaction Buffer	400 µL
C4 Antiserum	40 µL

Note: All reagents should be equilibrated to room temperature before use.

8.1 Switch NEPHSTAR on, choose Manual mode.
8.2 Enter chemistry number. Enter the chemistry number of C4 Kit (C4 = 34). If C4 assay has never been performed on the instrument before, please swipe card when “please swipe card” is displayed.
8.3 The assay name and the lot of reagent will be displayed. Press ENTER if the lot number is identical to that printed on the card or the kit label, otherwise swipe card to update the curve data stored in Nephstar.
8.4 Enter sample ID. Press number keys to enter the sample ID; or press ENTER to accept the currently displayed sample ID.
8.5 Enter sample dilution: 11. Accept the default sample dilution factor by pressing ENTER, otherwise press number keys to alter the dilution factor.
8.6 Dilute samples or controls using the Sample Diluent supplied with Nephstar Accessory Pack (Cat No.: NSAS200). The default dilution factor for C4 assay is 11 (e.g. 400 µL of Sample Diluent + 40 µL of sample or control).

8.7 Prepare one cuvette for each sample to be assayed. Place a stirring bar to the cuvette using forceps, then add 40 μL of diluted sample to the bottom of the cuvette.
8.8 Place the cuvette into the chamber and press it down slightly until it reaches the bottom of the chamber. The cuvette will be detected automatically.
8.9 Add 400 μL of C4 Reaction Buffer and then 40 μL of C4 Antiserum into the cuvette with common pipettes. Press "NUM LOCK" button. The reaction will be started immediately. The assay begins after blanking and the result will be printed out 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. To change the sample ID, press BACK twice and tap in the right number.
8.11 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 accordance with good laboratory practice, users should run control with every batch of samples. Results of control should fall in the validity range provided along with the control.

10. Limitations

Sample concentration of less than 16 g/L will not result in antigen excess. If the concentration is higher than 16 g/L, the results will be misleadingly low. If this is suspected, further dilute the sample to 1/55 (160 μL of Sample Diluent + 40 μL of 1/11 diluted sample), and reperform the assay.

11. Reference Range

11.1 The expected reference range of serum C4 of healthy adults is: 0.1 - 0.4 g/L. The expected reference range may vary with age, gender, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.
11.2 Diagnosis and treatment should not only depend on determination of C4 alone. The clinical symptoms and other laboratory findings of patients should always be taken into consideration.

12. Performance

12.1 Precision: CV ≤ 10%
12.2 Accuracy: Relative deviation within 20%
12.3 Linear range: within 0.06 – 2.20 g/L, the correlation coefficient ≥ 0.99, linear deviation within 20%

13. Caution and Warning

13.1 The reagents are only for *in vitro* diagnostic use.
13.2 The reagents can be used only by trained personnel with good laboratory practice and the stated procedure should be followed strictly.
13.3 All antiserums have been tested to be HBsAg negative HIV (1&2) antibody negative, HCV Ab negative. However, none of the testing methods can assure the absolute absence of infectious agents in blood products so please be sure to handle the blood products such as controls and antiserums as potentially infectious materials.
13.4 All reagents of the kit contain sodium azide as preservative. Take caution when handling them. Avoid ingesting or contacting of the reagents with skin or mucosa. If the contact occurs wash with large amount of water and seek medical advice. Azide may form explosive metal compounds in lead or copper plumbing. When disposing these reagents, be sure to flush with large amount of water to avoid accumulation of azide.
13.5 All components of this kit are exclusive for Nephstar. Reagents of different lots are not interchangeable. The results may not be reliable if reagents from different lots are mixed or used together.

14. Reference

Milford Ward, A., P. G. Riches, and A. M. Smith. PRU handbook of clinical immunochemistry. (1999).

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