

NEPHSTAR® Antithrombin III (AT3) Kit

Catalog No. **DK019**

1. Intended Use

This product is used on NEPHSTAR® protein analysis system for quantitative determination of human Antithrombin III(AT-III, AT3) in plasma(sodium citrate as anticoagulant) as an aid in diagnosis of abnormal AT-III metabolism.

2. Summary

Antithrombin III is a single-chain glycoprotein of molecular weight 58,000 produced by the liver and consists of 432 amino acids. The reactivity of AT-III is regulated by combination with its activator heparin. It is an inhibitor of thrombin, Factor Xa, Factor VIIa, Factor IXa, Factor XIa, Factor XIIa. There is more AT3 in blood than prothrombin. Blood is able to clot only because the reaction of the inhibitor with thrombin is much slower than the action of thrombin on fibrinogen.

Antithrombin III is mainly involved in the function of anticoagulant system. Decreased levels of AT-III are found in nephrotic syndrome, DIC, deep vein thrombosis, hypercoagulability syndrome, oral contraceptives, pulmonary embolism, direct hepatotoxicity, extra-corporeal circulation and L-asparaginase administration. Increased levels of AT3 would not cause pathological changes in general.

3. Test Principle

Immunonephelometry is applied. This method involves measuring the light scattered by insoluble complexes formed by reaction between specific protein in samples and its respective antiserum, and the amount of scattered light is directly proportional to the concentration of the protein under condition that antiserum is in excess. Concentrations are automatically calculated by reference to a calibration curve stored in the instrument.

4. Kit Components

Code	Name	Volume/Quantity
DA019	AT3 Antiserum	1×2.0 ml
DB019	AT3 Reaction buffer	1×25.0 mL
DC019	AT3 Magnetic card	1
DM019	AT3 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°C 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°C and the buffer at 18-25°C 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 fresh plasma .The plasma may be stored at 2-8°C for 48 hours, otherwise freeze at -20°C or below; do not freeze and thaw plasma more than once. Sample dilutions should be freshly prepared on the day of assay. Testing of the following types of plasma 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 (undiluted)	10ul
AT3 Reaction Buffer	400ul
AT3 Antiserum	40ul

- 8.1 Switch NEPHSTAR on.
- 8.2 Enter chemistry number. Enter chemistry number of AT3 kit (AT3=19). If AT3 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)if necessary. The default dilution scheme for AT3 assay is 1/1 (undiluted) .
- 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 10uL of 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: 1. 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 10uL of 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 AT3 reaction buffer and 40 uL AT3 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. 1/5 (160µL sample diluent + 40µL sample) . Accordingly the sample dilution should be

altered to 1/5 (press BACK and then the number keys to alter the sample dilution) .

- 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. Sensitivity and measuring range

The sensitivity limit is 0.06g/L and the upper limit is 0.75g/L when the default dilution scheme is applied. The sensitivity limit is 0.30g/L when samples are diluted at 1/5.

11. Antigen Excess

Sample concentration of less than 2g/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 1/5 (160uL sample diluent + 40uL undiluted sample).

12. Reference Range

12.1 Normal range of AT3 concentration of healthy adult is: 0.22-0.39g/L. We recommend local reference ranges are produced.

12.2 Diagnosis and treatment can not only depend on determination of AT3. 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:

AT3 (g/L)	CV (%)
0.2	4.0
0.46	3.5

14. Correlation Study

A correlation study is performed on 20 clinical serum samples using this kit on NEPHSTAR and Behring AT3 reagent on BNII. The linear regression equation and correlation equation got as showed below demonstrate a good correlation between the two methods:

$$Y=0.97X+0.013$$

$$(Y=\text{NEPHSTARTM AT3}, X=\text{BNIITM AT3})$$

Correlation coefficient $r=0.99$

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. References

16.1. 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.

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

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

16.4 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.



Goldsite Diagnostics Inc.

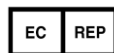
3rd Floor, Technology Building Annex

7th Industrial Road, Shekou

Shenzhen, P.R.China, 518067

Tel: 86 755 26890807

Fax: 86 755 26890799



CMC MEDICAL DEVICES & DRUGS, S.L.

C/ Horacio Lengo No 18, CP 29006, M áaga-Spain

