NEPHSTAR®
Serum Amyloid A（SAA）Kit

Catalog No. **BK052**

1. Intended Use
This product is used on NEPHSTAR® protein analysis system for quantitative determination of human Serum Amyloid A (SAA) in serum.

2. Summary
SAA is mainly derived from the constitutive expression of C-SAA in hepatocytes. SAA has two forms, one is the acute phase SAA is A-SAA, one is constitutive SAA, C-SAA, the general inflammatory response mainly affects the A-SAA. After the body is stimulated (inflammation, infection, injury, cancer, etc.) produce a series of cytokines, such as interleukin 1 (IL-1), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-alpha), A-SAA is upregulated by cytokines and becomes the major SAA in vivo at this time. SAA is an acute phase reaction protein that belongs to a heterogeneous class of proteins in the apolipoprotein family, Its concentration is a sensitive indicator of the early inflammatory response to infectious diseases, helping to diagnose inflammation, assessing its activity, monitoring its activity and treatment. SAA has been used successfully for clinical diagnosis and monitoring of a variety of infections and diseases, including infections caused by bacteria, fungi, and viruses, hand-foot-and-mouth disease, Neonatal early septicemia; Measurements of SAA are especially useful in distinguishing viral from bacterial infections. The acute nature of many diseases in which SAA is relevant for diagnosis and monitoring requires a rapid, easily interpreted, quantitative test, which comes true with this kit.

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
BA052	SAA Antiserum	1×2.0 ml
BB052	SAA Reaction buffer	1×25.0 mL
BM052	SAA Control	1*0.5ml
DY05655	Diluent1	1*55ml
BC052	SAA Magnetic card	1
	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 200-1000uL(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. Store serum at 2-8 °C for up to 7 days; -20 ℃ Store for not more than 30 days, 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（1/41）	40ul
SAA Reaction Buffer	400ul
SAA Antiserum	40ul

8.1 Switch NEPHSTAR on.

8.2 Enter chemistry number. Enter chemistry number of SAA kit (SAA=52). If SAA 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 serum samples or controls using NEPHSTAR Sample Diluent supplied in NEPHSTAR Accessory pack (Cat. No: DK110）. The default dilution scheme for SAA assay is 1/41 (e.g.800uL 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: 41. 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 SAA reaction buffer and 40 uL SAA 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 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 3mg/L and the upper limit is 200mg/L when the default dilution scheme is applied.

11. Limitation

When the sample concentration is higher than reportable range 550mg/L, it's not suggested to increase dilution for further testt. Sample concentration of less than 1000mg/L will not result in antigen excess. If the concentration is higher than 1000mg/L, the results will be misleadingly low.

12. Reference Range

12.1 Normal range of SAA concentration is: <10.08mg/L. We recommend each laboratory sets up its own reference range according to its patient group.

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

SAA（mg/L）	CV（%）
25.3	5.42
142	4.38

14. Correlation Study

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

Y=1.017X+2.37

(Y= NEPHSTAR® SAA, X=BNII SAA)

Correlation coefficient r=1.038

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. A study on serum amyloid substance A [J]. Journal of modern laboratory medicine, 2010, 25(4): 10-12.

2. Malle E, De Beer FC. Human serum amyloid A (SAA) protein: a prominent acute-phase reactant for clinical practice. Eur J Clin Invest. 1996 Jun; 26 (6):427-35.

3. CUNNANE G, GREHAN S, et al. Serum amyloid A in the assessment of early inflammatory arthritis [J]. J Rheumatol, 2000, 27(1): 58-63.

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