MININEPH™ HUMAN APOLIPOPROTEIN B KIT

For *in vitro* diagnostic use

Product Code: ZK086.R

Product manufactured by:
The Binding Site Group Ltd, 8 Calthorpe Road, Edgbaston, Birmingham, B15 1QT, UK
www.bindingsite.co.uk
Telephone: +44 (0)121 456 9500
Fax: +44 (0)121 456 9749
e-mail: info@bindingsite.co.uk

CE

1 INTENDED USE

This kit is designed for the *in vitro* measurement of human Apolipoprotein B (Apo B) in serum using the MININEPH or MININEPHPLUS* as an aid in the diagnosis and treatment of atherosclerosis and coronary artery disease.

**The MININEPHPLUS analyser is not available in the USA.*

2 SUMMARY AND EXPLANATION

Apolipoprotein B (Apo B) is the major protein in very low density, intermediate density and low density lipoproteins. It has a molecular weight of 549kDa as measured by SDS-PAGE (ref 1). The normal serum concentration is approximately 0.8g/L. It is mainly synthesized in the liver and is the physiological ligand for the LDL (Apo B,E) receptor. Its involvement with cholesterol transport means that increased levels of Apo B are strongly correlated with increased risk of atherosclerosis and coronary artery disease (refs 2-4).

3 PRINCIPLE OF THE ASSAY

The determination of soluble antigen concentration by nephelometric methods involves a reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed, a portion of the light is scattered and detected by a photodiode. The amount of light scattered is directly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

4 REAGENTS

4.1 MININEPH HUMAN APOLIPOPROTEIN B ANTISERUM
This has been adsorbed to monospecificity for Apo B and is supplied in a stabilised liquid form. It contains 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.

4.2 MININEPH APOLIPOPROTEIN B SWIPE CARD
This is encoded with details of the reaction curve specific to the respective lot of antiserum. This card is antiserum lot specific and must be used only with this lot of antiserum. The curve on this card has been prepared using secondary calibration materials that have been calibrated against the WHO international reference preparation SP3-07.

4.3 MININEPH APOLIPOPROTEIN B BUFFER
For use with this lot of Apo B reagent only. Contains 0.099% sodium azide as a preservative.

4.4 MININEPH HUMAN APOLIPOPROTEIN B HIGH AND LOW CONTROLS
These consist of pooled normal human serum and are supplied in lyophilised form. Reconstitute each vial with 0.5mL distilled water and allow to stand for 15 minutes prior to use. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. The acceptable ranges of Apo B concentrations are stated on the Quality Control Certificate included in the kit. The lot number quoted on the Quality Control Certificate should be identical to the kit lot number.

5 CAUTION

All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either approved by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained persons for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times. Results are likely to be invalid if parameters other than those stated in these instructions are used.

Reagents from different batch numbers of kits are NOT interchangeable. If large numbers of tests are performed care should be taken to ensure that all reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kits should be stored at 2-8°C and can be used until the expiry date given on the kit box label. DO NOT FREEZE. The buffer should be allowed to equilibrate to room temperature prior to use. Once opened the antiserum and controls should be stored at 2-8°C and the buffer at room temperature. Opened antisera and buffer, and reconstituted controls are stable for 4 weeks when stored as recommended. The On-Board Buffer 1 should be stored at room temperature. Opened On-Board Buffer 1 is stable for 4 weeks when stored as recommended.

7 SPECIMEN COLLECTION AND PREPARATION

Use serum samples. Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. Sera may be stored at 2-8°C for up to two days, otherwise aliquot and 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. Some types of sera are not suitable for MININEPH assay – see section 10.1.

8 METHODOLOGY

8.1 MATERIALS PROVIDED

8.1.1 1 x 1mL *MININEPH Human Apolipoprotein B Antiserum*
8.1.2 1 x 14mL *MININEPH Apolipoprotein B Buffer*
8.1.3 1 x 0.5mL *MININEPH Human Apolipoprotein B High Control*
8.1.4 1 x 0.5mL *MININEPH Human Apolipoprotein B Low Control*
8.1.5 Magnetic swipe card containing lot specific calibration information
8.1.6 Quality Control Certificate
8.1.7 Instruction leaflet

8.2 MATERIALS REQUIRED BUT NOT PROVIDED (MININEPH)

8.2.1 MININEPH instrument (AD200)
8.2.2 MININEPH printer (AD210) (optional)
8.2.3 MININEPH reagent accessory pack (ZK500.R)
8.2.4 Electronic pipette (e.g. AD205)
8.2.5 A range of pipettes capable of dispensing 5 – 1000µL
8.2.6 Equipment for the collection and preparation of test samples
8.2.7 Distilled water

8.3 MATERIALS REQUIRED BUT NOT PROVIDED (MININEPHPLUS)

8.3.1 MININEPHPLUS instrument (AD500 .C/D/E)
8.3.2 MININEPHPLUS PRINTER (AP1310DPK1T63) (optional)
8.3.3 Bar Code Reader (optional)
8.3.4 MININEPH reagent accessory pack (ZK500.R)
8.3.5 Pipette (5-1000µL)
8.3.6 Equipment for the collection and preparation of test samples
8.3.7 MININEPHPLUS On-Board Buffer 1 (SN107)
8.3.8 Pipette tips for use with the MININEPHPLUS – refer to MININEPHPLUS User Guide.

8.4 TEST PROCEDURE - MNINEPH ANALYSER

8.4.1 Summary of reagent volumes added to the cuvette:

Reagent	Volume added
Sample (1/30 dilution)	40µL
MININEPH Apolipoprotein B Buffer	400µL
MININEPH Hu Apolipoprotein B Antiserum	40µL

8.4.2 Switch the analyser and printer (if attached) on.
8.4.3 *Enter chemistry number*. Enter the chemistry number (Apo B = 86) and press **enter**.
8.4.4 *Swipe chemistry card*. This message will only be displayed if this chemistry has never been used before or you wish to change antiserum lot number. Pass the swipecard through the swipecard reader moving from the front of the instrument to the back. The magnetic strip should be at the bottom facing left.
8.4.5 *Check reagent lot number*. Press **enter.**
8.4.6 *APO B lot xxxx. OK? 1=Y 2=N*. Compare the details displayed with those on the antiserum label. If the lot number displayed is identical to that printed on the antiserum vial, select YES (**press 1**) and continue to step 8.4.7. If the vial lot number is different from that displayed select NO (**press 2**) and return to step 8.4.4 to allow the details of the correct batch to be entered.
8.4.7 Prepare dilutions of controls and samples using the MININEPH Sample Diluent supplied in the MININEPH Reagent Accessory Pack (ZK500.R). The recommended sample dilution for Apo B is 1/30 (e.g. pipette 20µL sample into a sample dilution tube and add 580µL of MININEPH Sample Diluent).
8.4.8 Prepare one MININEPH cuvette for each sample to be assayed. Using the forceps provided with the MININEPH place a stirring bar in each cuvette and then using a pipette add 40µL of diluted sample carefully to the bottom of each cuvette.
8.4.9 *Enter sample ID*. Enter an identity code (e.g. 1) for the first sample to be assayed then press **enter** to continue (refer to user manual for choice of identity codes).
8.4.10 *Sample dilution 1/30.* Accept the recommended dilution by pressing **enter,** or type in a new dilution factor if an alternative dilution is to be used.
8.4.11 *Place cuvette in chamber*. Place a cuvette containing a stirring bar and 40µL of diluted sample in the cuvette chamber. Press the cuvette down gently until it reaches the bottom of the chamber. The cuvette will be detected automatically.
8.4.12 *Add reagent.* Fill an electronic pipette with 400µL of MININEPH Apo B Buffer and 40µL of MININEPH Hu Apo B Antiserum and dispense its contents into the cuvette. The MININEPH will detect the addition followed by movement of the stirring bar and the assay will begin. It is not necessary to press enter. After a 10 second blanking time the assay will take 210 seconds to complete, the result will then be displayed and printed automatically (if a printer is connected).
8.4.13 On completion of the assay remove the cuvette and press **enter** to perform the next assay.
8.4.14 If the instrument indicates the result is higher than the intended measuring range, reassay the sample at a higher dilution of 1/150 (160µL MININEPH Sample Diluent + 40µL sample diluted 1/30). The sample dilution should be entered as 1/150 (see section 8.4.10).
8.4.15 If the instrument indicates the result is lower than the intended measuring range, reassay the sample at a lower dilution of 1/5 (160µL MININEPH Sample Diluent + 40µL sample). The sample dilution should be entered as 1/5 (see section 8.4.10).
8.4.16 When all assays for the chosen chemistry have been completed press escape (**esc**) and select the chemistry number for the next set of assays.

8.5 TEST PROCEDURE FOR MININEPHPLUS ANALYSER

8.5.1 Summary of reagent volumes added to the cuvette:

Reagent	Volume added
Sample (1/30 dilution)	40µL
MININEPH Apolipoprotein B Buffer	400µL
MININEPH Hu Apolipoprotein B Antiserum	40µL

8.5.2 Ensure that an empty waste pot is placed at the back of the MININEPHPLUS.
8.5.3 Attach a new pipette tip on the end of the MININEPHPLUS hand held pipette.
8.5.4 Check there is sufficient On-Board buffer 1 (SN107) in the drawer. There needs to be at least 10mL. *Refer to the* MININEPHPLUS *User Guide for instructions on replenishing the buffer.*
8.5.5 Switch on the analyser and printer (if attached).
8.5.6 *Enter chemistry number.* Enter the chemistry number (Apo B = 86) and press **enter**.

8.5.7 *Swipe chemistry card.* This message will only be displayed if this chemistry has never been used before or when changing antiserum lot number. Pass the swipecard through the swipe card reader moving in a left to right direction across the front of the analyser. The magnetic stripe facing upwards.

8.5.8 *Check reagent lot number. Press* ***enter****.*

8.5.9 *APO B lot xxxx. OK? 1=Y 2=N.* Compare the details displayed with those on the antiserum label. If the lot number displayed is identical to that printed on the antiserum vial, select YES (**press 1**) and continue to step 8.5.10. If the vial lot number is different from that displayed select NO (**press 2**) and return to step 8.5.7 to allow the details of the correct batch to be entered.

8.5.10 *Prime?* 1=Y 2=N. Prime the analyser to expel air bubbles in the plastic tube leading from the On-board buffer bottle to the hand-held pipette. This is done by pressing button **1** when prompted. Excess On-board buffer will be expelled into the waste pot. When priming has finished press **2.** Note that a prime will always be performed when starting a T1 assay that follows a T2 assay

8.5.11 *Pipette Y/N: Block Y/N*. There is a short period when the MININEPHPLUS stabilises its temperature.

8.5.12 Prepare dilutions of controls and samples using the MININEPH Sample Diluent supplied in the MININEPH Reagent Accessory Pack (ZK500.R). The recommended sample dilution for Apo B is 1/30 (e.g. pipette 20µL sample into a sample dilution tube and add 580µL of MININEPH Sample Diluent).

8.5.13 Prepare one MININEPH cuvette for each sample to be assayed. Using the forceps provided with the MININEPHPLUS place a stirring bar in each cuvette and then using a pipette add 40µL of diluted sample carefully to the bottom of each cuvette.

8.5.14 *Enter sample ID.* Enter an identity code (e.g. 1) for the first sample to be assayed then press **enter** to continue (refer to user manual for choice of identity codes).

8.5.15 *Sample dilution 1/30.* Accept the recommended dilution by pressing **enter** or type in a new dilution factor if an alternative dilution is to be used.

8.5.16 *Place cuvette in chamber.* Place a cuvette containing a stirring bar and 40µL of diluted sample in the cuvette chamber. Press the cuvette down gently until it reaches the bottom of the chamber. The cuvette will be detected automatically.

8.5.17 *Supplementary buffer.* Using the MININEPHPLUS hand-held pipette, aspirate 400µL of MININEPH Apo B buffer.

8.5.18 *Air Gap.* Using the MININEPHPLUS hand-held pipette, aspirate an air gap.

8.5.19 *Aspirate Reagent* Using the MININEPHPLUS hand-held pipette, aspirate 40µL of MININEPH Human Apo B antiserum.

8.5.20 *Add Reagent.* Dispense the aspirated reagents into the cuvette. The stirring bar will rotate and the assay will begin. After a 10 second blanking time the assay will take 208 seconds to complete. The result will be displayed. Results will be automatically printed if a printer is connected.

8.5.21 If the instrument indicates the result is higher than the standard measuring range, reassay the sample at a higher dilution of 1/150 (160µL MININEPH Sample Diluent + 40µL sample diluted 1/30). The sample dilution should be entered as 1/150). The sample dilution should be entered as 1/150 (see section 8.5.15).

8.5.22 If the instrument indicates the result is lower than the standard measuring range, reassay the sample at a lower dilution of 1/5 (160µL MININEPH sample diluent + 40µL sample). The sample dilution should be entered as 1/5 (see section 8.5.15).

8.5.23 On completion of the assay remove the cuvette and press **enter** to perform the next assay.

8.5.24 When all assays for the chosen chemistry number have been completed press **esc** and select the chemistry number for the next set of assays.

8.5.25 Empty waste pot and discard the pipette tip from the hand held pipette.

8.6 QUALITY CONTROL

As with all good laboratory practice, users should run controls with every batch of samples.

9 INTERPRETATION OF RESULTS

9.1 Results are calculated by the instrument and displayed in g/L. If a printer is attached the result is automatically printed out together with the patient identification code and the sample dilution. Further calculations are not necessary.

9.2 When using the recommended sample dilution the approximate measuring range is 0.3 – 2.4g/L. The sensitivity limit is 0.06g/L when using a 1/5 dilution. Sample concentrations up to at least 12.5g/L will not result in antigen excess. Higher concentrations may give misleading results; if this is suspected, samples should be reassayed at a 1/150 dilution (160µL MININEPH Sample Diluent + 40µL sample diluted 1/30).

10 LIMITATIONS OF PROCEDURE

10.1 SPECIFIC TEST LIMITATIONS

10.1.1 Nephelometric assays are not suitable for measurement of highly lipaemic or haemolysed samples or samples containing high levels of circulating immune complexes (CICs) due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.

10.1.2 Diagnosis cannot be made and treatment must not be initiated on the basis of Apo B measurements alone. Clinical history and other laboratory findings must also be taken into account.

10.2 TROUBLE SHOOTING

Problem	Possible Causes(s)	Suggested Action(s)
Error message "Blank too high – reassay" displayed.	Very high analyte concentration.	Reassay sample at a higher dilution.
	Lipaemic, turbid or haemolysed samples.	Try alternative assay method.
Controls out of range.	Product deterioration.	Check expiry date.
	Operator error.	Repeat assay with the correct sample dilution.
Test sample giving unexpectedly low result.	Antigen excess.	Repeat assay at higher dilution. Check if the two results agree.

11 EXPECTED RESULTS

The following Apo B results were obtained with normal adult donor sera on the MININEPH. Concentrations are in g/L. We recommend local reference ranges are generated.

Number	Mean	Median	95 Percentile Range
100	0.87	0.88	0.43 – 1.40

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

12.1.1 Precision - MININEPH

Apo B Precision Summary						
	Intra batch		Day to day		Inter instrument	
	Mean g/L	CV% (n=20*)	Mean g/L	CV% (n=10**)	Mean g/L	CV% (n=10***)
Serum 1	1.75	2.83	1.50	4.46	1.73	3.77
Serum 2	0.45	5.15	0.85	4.19	0.44	8.17

12.1.2 Precision –MININEPHPLUS

Apo B Precision Summary				
	Mean g/L	Intra batch CV% (n=30^{+})	Day to day CV% (n=30^{++})	Inter instrument CV% (n=15^{+++})
Serum 1	1.68	3.22	3.41	3.10
Serum 2	0.80	5.29	6.79	4.53

**These data represent the coefficient of variation (CV) of twenty within-batch measurements at three analyte concentrations.*
*** Assays were performed at three different concentrations on ten separate occasions. The CV of the ten results at each concentration was calculated.*
**** Assays were performed at three different concentrations on each of ten instruments. The CV of the results at each concentration was calculated.*
$^{+}$ These data represent the average coefficient of variation (CV) of three within-batch measurements repeated ten times at each concentration.
$^{++}$ Ten within-batch measurements were performed on three separate occasions and the overall CV for the thirty results at each concentration calculated.
$^{+++}$ Assays were performed five times at each concentration on three instruments. The overall CV of the fifteen results at each concentration was calculated.

12.2 COMPARISON STUDY

12.2.1 MININEPH

A correlation study was performed on 100 normal serum samples using this kit on a MININEPH and a Behring Apolipoprotein B assay on a BN™A. The study demonstrated a good agreement yielding the following linear regression equation and correlation coefficient.

y = 0.924x - 0.05 g/L (y = MININEPH Apo B)

(x = BNA Apo B)

correlation coefficient r = 0.926

BN™ is a trademark Siemens Healthcare Diagnostics Inc.

12.2.2 MININEPHPLUS

30 normal adult sera were tested on the MININEPH and MININEPHPLUS. The study demonstrated a good agreement yielding the following Passing & Bablok equation and linear regression correlation coeffiecient:

y = 0.96x + 0.03 g/L (y = MININEPHPLUS Apo B)

(x = MININEPH Apo B)

correlation coefficient r = 0.993

13 REFERENCES

1. Haeberli, A. (1992) Human Protein Data© VCH Verlages mbH Weinheim
2. Rifai, N (1986) Lipoproteins and Apolipoproteins Composition, Metabolism and Association with Coronary Heart Disease. Arch Pathol Lab Med 110, 694-701.
3. Reinhart, R. A. *et al* (1990) Apolipoproteins A-1 and B as Predictors of Angiographically Defined Coronary Artery Disease, Arch Intern Med 150 1629-1633.
4. Kottke, B.A. et al (1986) Apolipoproteins and Coronary Artery Disease, Mayo Clin Proc 61 313-320.