MININEPH™ HUMAN IgA KIT

For in vitro diagnostic use

Product Code: ZK010.R

Product manufactured by: The Binding Site Group Ltd, 8 Calvary 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

FDA (USA) Information (for MININEPH analyser only)

Analyte ID: 2803

Test System: 61362

Complexity Cat: Moderate

MININEPH™ and MININEPH PLUS™ are registered trademarks of The Binding Site Group Limited (Birmingham, UK) in certain countries.

1 INTENDED USE

This kit is designed for the in vitro measurement of human IgA in serum using the MININEPH or MININEPH PLUS™ as an aid in the diagnosis of disorders associated with IgA deficiency. When using the recommended dilution the approximate measuring range is 0.37-5.94g/L. The sensitivity limit is 0.17g/L when using a 1/5 sample dilution.

*The MININEPH PLUS assay is not available in the USA.

2 SUMMARY AND EXPLANATION

IgA is the major immunoglobulin class of serum-mucosal secretions, part of the defence system for external body surfaces. The monomeric form is composed of two alpha heavy chains and two light chains. Two subclasses of IgA have been identified in humans, IgA1 and IgA2. Normal serum levels of IgA vary with age. Raised IgA serum levels are associated with breast feeding, chronic infections, liver disease and myeloma. Reduced levels may be associated with certain protein-losing conditions (refs 1, 2).

3 PRINCIPLE OF THE ASSAY

The determination of soluble antigen concentration by nephelometric methods involves a reaction with specific antisera to form insoluble complexes. When light is passed through the suspended complexes, 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 IgA antiserum

This has been adsorbed to monospecificity for IgA and is supplied in stabilised liquid form. It contains 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.

4.2 MININEPH IgA swipe card

This is encoded with details of the reaction curve specific to the respective lot of antiserum. This card is antisera lot specific and must be used only with this lot of antisera. The curve on this card has been prepared using secondary calibration materials that have been calibrated against DA470k.

4.3 MININEPH IgA buffer

For use with this lot of IgA reagent only. Contains 0.089% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.

4.4 MININEPH Human IgA high and low controls

These consist of pooled normal human serum and are supplied in stabilised liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. The acceptable ranges of IgA 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 (particular 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 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.

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 label box. DO NOT FREEZE. The IgA 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 antiserum, IgA buffer and controls are stable for 12 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 aspirated 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 then re-heat to room temperature. 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 2ml MININEPH Human IgA Antiserum

8.1.2 1 x 25mL MININEPH IgA Buffer

8.1.3 1 x 5.5mL MININEPH Human IgA High Control

8.1.4 1 x 5.5mL MININEPH Human IgA Low Control

8.2 Equipment for the collection and preparation of test samples

8.2.1 MININEPH instrument (AD200).

8.2.2 MININEPH reagent accessory pack (ZK500.R)

8.2.3 MININEPH PLUS PRINTER (AP1310DPK1T63) (optional)

8.2.4 Pipette (5-40μL)

8.2.5 Pipette (500μL)

8.2.6 Equipment for the collection and preparation of test samples

8.3 Materials required but not provided (MININEPHPLUS)

8.3.1 MININEPH instrument (AD200).

8.3.2 MININEPH printer (AD2210) (optional)

8.3.3 MININEPH reagent accessory pack (ZK500.R)

8.3.4 Equipment for the collection and preparation of test samples

8.3.5 Pipette tips for use with the MININEPHPLUS – refer to MININEPHPLUS User Guide.

8.4 Test procedure – MININEPH Analyser

8.4.1 Summary of reagent volumes added to the cuvette:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume added</th>
</tr>
</thead>
<tbody>
<tr>
<td>MININEPH IgA Buffer</td>
<td>40μL</td>
</tr>
<tr>
<td>MININEPH Hu IgA Antiserum</td>
<td>40μL</td>
</tr>
</tbody>
</table>

8.4.2 Switch the analyser and printer (if attached) on.

8.4.3 Enter chemistry number. Enter the chemistry number for the next set of assays.

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 swictheb card through the swicthebar 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. Pass the magnetic swipe card through the swicthebar reader moving from the front of the instrument to the back. The magnetic strip should be at the bottom facing left.

8.4.6 IG A lot xxx. OK? Try 2/IN. Compare the details displayed with those on the antiserum label. If the lot number displayed is identical to that printed on the antisera vial label then pass 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 IgA is 1/11 (e.g. using the electronic pipette dispense 400μL of sample diluent and 40μL of sample into a sample dilution tube).

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 the 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 1/11. 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 40μL of MININEPH IgA Buffer and 40μL of MININEPH Hu IgA 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. Press enter to print result. After a 10-second blanking time the assay will take 60 seconds to complete, the result will then be displayed and printed automatically (if a printer is connected).

8.4.13 If the instrument indicates the result is higher than the intended measuring range, reassay the sample at a higher dilution of 1/50 (980μL MININEPH sample diluent + 40μL sample). The sample dilution should be entered as 1/50 (see section 8.4.10).

8.4.14 If the instrument indicates the result is lower than the intended measuring range, reassay the sample at a lower dilution of 1/160 (MININEPH sample diluent + 40μL sample). The sample dilution should be entered as 1/160 (see section 8.4.10).

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

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:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample (1/11 dilution)</td>
<td>40μL</td>
</tr>
<tr>
<td>MININEPH IgA Buffer</td>
<td>40μL</td>
</tr>
<tr>
<td>MININEPH Hu IgA Antiserum</td>
<td>40μL</td>
</tr>
</tbody>
</table>

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 to the end of the MININEPH PLUS 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 MININEPH PLUS 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 (IgG = 10) and press enter.

8.5.7 Siphe pipette card. This message will only be displayed if this chemistry has never been used before or when changing antisemur lot number. Pass the swepethrough or siphe pipette card reader in a left to right direction across the front of the MININEPH PLUS with the magnetic strip facing upwards.

8.5.8 Check reagent lot number. Press enter.

8.5.9 IgG lot xxx. CW? 1=Y 2=N. Compare the details displayed with those on the antisemur label. If the lot number displayed is identical to that printed on the antisemur vial, select YES (press 1) and continue to step 8.5.12. If the lot number is different from that displayed select NO (press 2) and return to step 8.5.8 to allow the details of the correct batch to be entered.

8.5.10 Prime? 1=Y 2=N. Prime the analyser to expel bubbles in the plastic tubing 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 MININEPH PLUS stabilises its temperature.

8.5.12 Prepare dilutions of controls and samples using the MININEPH Sample Diluent supplied in the MININEPH Reagent Accessory Pack (PK5000.R). The recommended sample dilution for IgG is 1:11 (e.g. dispense 400μL of sample diluent and 40μL of sample into a sample dilution tube).

8.5.13 Prepare one MININEPH cuvette for each sample to be assayed. Using the forceps provided with the MININEPH PLUS 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 1/11. 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 cuvettes will be detected automatically.

8.5.17 Supplementary buffer. Using the MININEPH PLUS hand-held pipette, aspirate 400μL of MININEPH IgG buffer.

8.5.18 Air Gap. Using the MININEPH PLUS hand-held pipette, aspirate an air gap.

8.5.19 Aspirate Reagent. Using the MININEPH PLUS hand-held pipette, aspirate 40μL of MININEPH Human IgA antisemur.

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 55 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, reasay the sample at a higher dilution of 1/5 (980μL MININEPH sample diluent + 40μL sample). The sample dilution should be entered as 1/50 (see section 8.5.15).

8.5.22 If the instrument indicates the result is lower than the standard measuring range, reasay 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 enter to continue. If the instrument indicates the result is higher than the standard measuring range, or if the instrument indicates the result is lower than the standard measuring range, then press enter to perform a second assay.

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

8.5.26 Screen concentration. Select YES (press 1) to continue (refer to user manual for choice of identity codes).

1.2 Precision

12.1 Precision - MININEPH

<table>
<thead>
<tr>
<th>IgA Precision Summary</th>
<th>Mean</th>
<th>Intra batch CV% (n=30*)</th>
<th>Day to day CV% (n=30**)</th>
<th>Inter instrument CV% (n=30***)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>3.18</td>
<td>4.35</td>
<td>4.72</td>
<td>3.56</td>
</tr>
<tr>
<td>Serum 2</td>
<td>1.17</td>
<td>3.22</td>
<td>3.97</td>
<td>5.38</td>
</tr>
</tbody>
</table>

*These data represent the average coefficient of variation (CV) of 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.

***Twenty-five performed five times at each concentration on three instruments. The overall CV of the fifteen results at each concentration was calculated.

12.2 Comparison studies

12.2.1 MININEPH

A correlation study was performed on 118 normal and clinical serum samples using this kit on a MININEPH and a Behring IgA assay on a BN*TA analyzer. The study demonstrated a good agreement yielding the following linear regression equation and correlation coefficient.

\[ y = 0.915x + 0.089g/L \]  \( r = 0.975 \)

BN*TA is a trademark of Siemens Healthcare Diagnostics, Inc.

12.2.2 MININEPHPLUS

30 normal adult sera and 20 clinical adult sera were tested on the MININEPH and MININEPHPLUS. The study demonstrated a good agreement yielding the following Passing & Babcock equation and linear regression correlation coefficient:

\[ y = 1.07x + 0.016g/L \]  \( r = 0.994 \)

**REFERENCES**


2. Bradwell, AR (1995), IgG and IgA Subclasses in Disease. ISBN 0704416239 (available from The Binding Site Ltd.).


**EXPECTED RESULTS**

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

<table>
<thead>
<tr>
<th>Number</th>
<th>Mean</th>
<th>95 Percentile Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>1.85</td>
<td>0.86-3.20</td>
</tr>
</tbody>
</table>