MININEPH™ HUMAN MICROALBUMIN KIT

For *in vitro* diagnostic use

Product Code: ZK032.U.R

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1 INTENDED USE

This kit is designed for the *in vitro* measurement of human albumin in urine using the MININEPH or MININEPHPLUS* to aid in the diagnosis of renal disease. When using the recommended dilution the approximate measuring range is 15 – 235mg/L. The sensitivity limit is 15mg/L.

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

2 SUMMARY AND EXPLANATION

Early detection and treatment of nephropathy is important in preventing renal failure in insulin-dependent diabetics. Elevated urinary albumin concentration is a good indicator of glomerular damage in such patients. Increased albumin excretion is also a marker of future cardiovascular problems in non insulin-dependent diabetes mellitus and also occurs in other chronic conditions such as hypertension, malignancy and chronic obstructive airways disease (refs 1, 2).

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 MICROALBUMIN ANTISERUM
This has been adsorbed to monospecificity for albumin 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 MICROALBUMIN 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 evaluated against DA470k.

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

4.4 MININEPH HUMAN MICROALBUMIN 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 albumin 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 personnel 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, controls and buffer are stable for 8 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 freshly collected urine samples: these should be centrifuged prior to analysis to remove particulate matter. Sample dilutions should be freshly prepared on the day of assay. Some types of urine are not suitable for MININEPH assay – see section 10.1.

8 METHODOLOGY

8.1 MATERIALS PROVIDED

8.1.1 1 x 1mL *MININEPH Human Microalbumin Antiserum*
8.1.2 1 x 14mL *MININEPH Microalbumin Buffer*
8.1.3 1 x 0.5mL *MININEPH Human Microalbumin High Control*
8.1.4 1 x 0.5mL *MININEPH Human Microalbumin 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 Pipette (5 - 40µL)
8.2.6 Equipment for the collection and preparation of test samples

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 FOR MININEPH ANALYSER

8.4.1 Summary of reagent volumes added to the cuvette:

Reagent	Volume added
Sample (undiluted)	40µL
MININEPH Microalbumin Buffer	400µL
MININEPH Hu Microalbumin Antiserum	40µL

8.4.2 Switch the analyser and printer (if attached) on.
8.4.3 *Enter chemistry number*. Enter the chemistry number (Microalbumin = 11) 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 *MRALB 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 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 sample carefully to the bottom of each cuvette.
8.4.8 *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).
8.4.9 *Sample dilution 1/1.* Accept the recommended dilution by pressing **enter,** or type in a new dilution factor if an alternative dilution is to be used.
8.4.10 *Place cuvette in chamber*. Place a cuvette containing a stirring bar and 40µL of sample in the cuvette chamber. Press the cuvette down gently until it reaches the bottom of the chamber. The cuvette will be detected automatically.
8.4.11 *Add reagent.* Fill an electronic pipette with 400µL of MININEPH Microalbumin Buffer and 40µL of MININEPH Hu Microalbumin 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 180 seconds to complete, the result will then be displayed and printed automatically (if a printer is connected).
8.4.12 If the instrument indicates the result is higher than the intended measuring range, reassay the sample at a dilution of 1/11 (400µL MININEPH Sample Diluent + 40µL sample). The sample dilution should be entered as 1/11 (see section 8.4.9).
8.4.13 On completion of the assay remove the cuvette and press **enter** to perform the next assay.
8.4.14 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 (undiluted)	40µL
MININEPH Microalbumin Buffer	400µL
MININEPH Hu Microalbumin 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 (Microalbumin = 11) 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 should be facing upwards.
8.5.8 *Check reagent lot number*. Press **enter**.
8.5.9 *MRALB 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 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 sample carefully to the bottom of each cuvette.

8.5.13 *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).

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

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

8.5.16 *Supplementary buffer.* Using the MININEPHPLUS hand-held pipette, aspirate 400µL of MININEPH Microalbumin buffer.

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

8.5.18 *Aspirate Reagent* Using the MININEPHPLUS hand-held pipette, aspirate 40µL of MININEPH Human Microalbumin antiserum.

8.5.19 *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 178 seconds to complete. The result will be displayed. Results will be automatically printed if a printer is connected.

8.5.20 If the instrument indicates the result is higher than the intended measuring range, reassay the sample at a dilution of 1/11 (400µL MININEPH Sample Diluent + 40µL sample). The sample dilution should be entered as 1/11 (see section 8.5.14).

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

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

8.5.23 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. Controls included in this kit should be treated in the same way as samples.

9 INTERPRETATION OF RESULTS

9.1 Results are calculated by the instrument and displayed in mg/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 The assay range is limited to that stated under Intended Use. Sample concentrations up to at least 940mg/L will not result in antigen excess, higher concentrations may give misleading results. We recommend users obtain approximate results prior to analysis using urine dipsticks. Samples should then be diluted appropriately to fall within the MININEPH measuring range.

10 LIMITATIONS OF PROCEDURE

10.1 SPECIFIC TEST LIMITATIONS

10.1.1 Microbially contaminated or turbid samples may not be suitable for nephelometric measurements and should not be used unless they have been centrifuged or prepared in some appropriate manner. An alternative assay method, e.g. radial immunodiffusion, is recommended if background turbidity cannot be removed.

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

10.2 TROUBLE SHOOTING

Problem	Possible cause(s)	Suggested action(s)
Error message "Blank too high – reassay" displayed.	Very high analyte concentration. Turbid samples.	Reassay sample at a higher dilution. Try alternative assay method.
Controls out of range.	Product deterioration. Operator error.	Check expiry date. 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 albumin results were obtained with normal adult donor urine samples on the MININEPH. Concentrations are in mg/L. We recommend local reference ranges are generated.

Sample Type	Number of samples	Range mg/L
Normal Urine	18	All samples <14.9

12 PERFORMANCE CHARACTERISTICS

12.1 PRECISION

12.1.1 Precision - MININEPH

Microalbumin precision summary				
	Mean mg/L	Intra batch CV% (n=30*)	Day to day CV% (n=30**)	Inter instrument CV% (n=15***)
Sample 1	160	2.64	4.66	4.15
Sample 2	36.0	2.23	4.20	3.15

12.1.2 Precision –MININEPHPLUS

Microalbumin precision summary				
	Mean mg/L	Intra batch CV% (n=30*)	Day to day CV% (n=30**)	Inter instrument CV% (n=15***)
Sample 1	117.1	3.43	4.30	2.58
Sample 2	29.3	5.17	5.66	5.11

**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

12.2.1.1 A correlation study was performed on 25 clinical urine samples (range 15 – 3311mg/L) using this kit on a MININEPH and Binding Site RID plates. The study demonstrated a good agreement yielding the following linear regression equation and correlation coefficient.

y = 1.03x + 56mg/L (y = MININEPH Microalbumin)

(x = RID Albumin)

correlation coefficient r = 0.994

12.2.1.2 A correlation study was performed on 18 normal urine samples using this kit on the MININEPH and a competitor assay on the Siemens BN™A. The study showed good agreement between the two methods.

MININEPH Microalbumin: All samples <14.9mg/L

BNA Microalbumin assay: <1.9 – 11.6mg/L

BN™A is a trademark of Siemens Healthcare Diagnostics Inc.

12.2.2 MININEPHPLUS

A correlation study was performed on 15 normal urine samples using this kit on the MINIENPH and MININEPHPLUS. The study demonstrated equivalence between the two analysers, with a maximum difference of <13%.

13 REFERENCES

1. Gosling P (1995). Microalbuminuria: a marker of systemic disease. Br. J. Hospital Medicine, 54, 285-290.
2. Milford Ward A, Riches PG, Fifield R and Smith AM (Eds) (1999) PRU Handbook of Clinical Immunochemistry. Publ. PRU Publications, Sheffield, UK.