Human Microalbumin Kit for use on SPA^{PLUS}®

For *in vitro* diagnostic use

Product Code: NK032.U.S

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1 INTENDED USE

This kit is designed for the *in vitro* measurement of human albumin in urine using the Binding Site SPA^{PLUS} turbidimetric analyser to aid in the diagnosis of renal disease. This test should be used in conjunction with other laboratory and clinical findings.

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

The determination of soluble antigen concentration by turbidimetric methods involves the reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed a portion of the light is transmitted and focused onto a photodiode by an optical lens system. The amount of transmitted light is indirectly 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 Human microalbumin antiserum: This antiserum is monospecific for albumin and is supplied in stabilised liquid form. It contains 0,099% sodium azide, 0.1 % EACA, 0.1% EDTA and 0.01% benzamidine as preservatives.

4.2 Calibrator and Controls: These consist of pooled 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 concentration of albumin given on the quality control certificate has been obtained by comparison with the DA470k international reference material.

4.3 Reaction Buffer: Containing 0.099% sodium azide as a preservative.

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 cleared 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 the reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kit should be stored at 2-8°C and can be used until the expiry date shown on the kit box label. DO NOT FREEZE. The Human Microalbumin Antiserum, Reaction Buffer, calibrators, and controls may be stored for up to three months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. The Human Microalbumin Antiserum and Reaction Buffer may be stored, uncapped, on the SPA^{PLUS} analyser for up to 30 days, provided that the main power switch (located at the rear of the left hand panel) is left switched on.

7 SPECIMEN COLLECTION AND PREPARATION

Use freshly collected urine samples: these should be centrifuged prior to analysis to remove particulate matter. Some types of urine are not suitable for the SPA^{PLUS} assay – see section 10.1.

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 100 Tests *Human Microalbumin Antiserum SPA^{PLUS}*
8.1.2 1 x *Human Microalbumin SPA^{PLUS} Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1.5mL *Human Microalbumin SPA^{PLUS} High Control*
8.1.4 2 x 1.5mL *Human Microalbumin SPA^{PLUS} Low Control*
8.1.5 1 x 100 Tests *Microalbumin Reaction Buffer SPA^{PLUS}*

8.2 Materials required but not provided

8.2.1 Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
8.2.2 A fully operational and equipped SPA^{PLUS} analyser.
8.2.3 Current analyser operating instructions: SPA^{PLUS} Reference Guide, Insert Code FIN012.
8.2.4 Sample Diluent (99: Dil 1) Product Code: SN080.S
8.2.5 SPA^{PLUS} Weekly Wash Protocol and Bottles, Product Code IK050.S

8.3 Reagent preparation

Before loading, gently mix by inversion ensuring no foam or bubbles are generated or remain on the surface as these may interfere with reagent aspiration.

8.4 Test procedure

The user should be familiar with the operation of the SPA^{PLUS} analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the manufacturer's instructions and the assay protocol entered as described below.
For full details of analyser operation refer to the SPA^{PLUS} Reference Guide (FIN012) supplied with the analyser.

8.4.1 Test parameters

8.4.1.1 Assay parameters are entered into item number 36

Item Name 36 MALB
DATA INFORMATION
Units mg/L
Decimals 3

ANALYSIS
Type End ▼

Main W.Length 1 340 ▼
Sub W.Length ▼
Method

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION
Type Spline 1 ▼
Standard
1 # 4 #
2 # 5 #
3 # 6 #
Auto Fill

NORMAL RANGE

	MALE LOW	MALE HIGH	FEMALE LOW	FEMALE HIGH
Serum	[ ]	[ ]	[ ]	[ ]
Urine	[ ]	[ ]	[ ]	[ ]
Plasma	[ ]	[ ]	[ ]	[ ]
CSF	[ ]	[ ]	[ ]	[ ]
Dialysis	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]

Page : 1 Print Hard Copy Next Page Save Return

Item Name 36 MALB

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 17
REAGENT1 VOL 180 µL
REAGENT2 VOL 20

Third mix ● OFF ○ ON
R1 Blank ● Water – Blank

DATA PROCESS
READ
START END
MAIN 53 54
SUB 30 31

ABSORBANCE LIMIT
LOW -3.0
HIGH 2.0

FACTOR
Blank correction *
ENDPOINT LIMIT 2.0
LINEAR CHECK (%) 0

Reaction Check
○ ON ● OFF
CHECK POINT
LOW -3
HIGH 3

DILUTION
Diluent ● 99: Dil 1 ○ 100: Dil 2
Pre Dilution Rate ▼
Auto Rerun Dilution Rate High 10 ▼
Auto Rerun Dilution Rate Low ▼

MONITOR
0 LEVEL SPAN 1
SPAN 3.0

PROZONE CHECK
START END LIMIT (%) Min dOD 0
FIRST [ ] [ ]
SECOND [ ] [ ] [ ] ○ Low ● High
THIRD [ ] [ ] [ ] ○ Low ● High

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*Automatically calculated

Item Name 36 MALB

Auto Rerun SW
● On ○ Off

Auto Rerun Range (Result)
○ On ● Off ● On ○ Off
Lower Higher
Serum Cal 1 # Cal 6 #
Urine
Plasma
CSF
Dialysis
Other

Bottle Size (ml)
24 Items 36 Items
Reagent1 60 Reagent1 0
Reagent2 R1 19.0 Reagent2 R1 0
Reagent2 R2 3.0 Reagent2 R2 0

Auto Rerun Condition (Absorbance)

Absorbance Range
Lower ○ On ● Off
Higher ● On ○ Off

Prozone Range ○ On ● Off

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The calibrator (Standard #) values are found in the Quality Control Certificate (SIN212.QC). Calibrator values on **Page 1** should be entered in ascending order, i.e. the lowest value first. The analyser will automatically calculate and enter the correct measuring ranges on item pages 3and 4 providing the Autofill button is pressed after typing the value for calibrator 6 on page 1. View Item parameter pages 3 and 4 to ensure correct value entry.

8.4.2 Special wash procedure for urine testing

Sample carry-over may occur from microalbumin to Freelite assays. To protect against this, Freelite and microalbumin samples must be run in separate batches. After running microalbumin samples the onboard cuvettes must be cleaned using the SPAPLUS Weekly Wash Protocol before they can be re-used with Freelite samples. Full instructions, parameters and bottles of wash fluid are supplied in SPAPLUS Weekly Wash Protocol and Bottles (IK050.S).

8.5 Measuring range

The approximate measuring range of the microalbumin assay is shown in the table below.

SPAPLUS analyser dilution	Approximate range (mg/L)
1/1 (neat)	**11-344**
1/10	110-3440

For samples measuring over 3440mg/L the following dilution should be used:

Overall dilution	Analyser dilution	Manual pre-dilution	Approximate range (mg/L)
1/50	1/1	1/50*	550-17200

* Make manual pre-dilution of 1/50 by taking 20µL of sample and add 980µL system sample diluent to achieve an initial 1/50 dilution. Present the 1/50 diluted sample for analysis. Multiply the result x 50.

9 QUALITY CONTROL

9.1 At least two levels of appropriate control material should be tested a minimum of once a day. In addition, controls should be tested after calibration, with each new lot of reagent and after specific maintenance or troubleshooting steps described in the SPAPLUS Operation Manual.

9.2 Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure. Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to the local technical support organisation.

9.3 The concentrations of the controls provided are stated on the accompanying QC certificate (SIN212.QC). Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.

10 LIMITATIONS

10.1 Turbidimetric assays are not suitable for measurement of lipaemic, haemolysed, turbid or microbially contaminated samples due to the unpredictable degree of non-specific scatter these sample types may generate. All samples must be centrifuged prior to assay. Unexpected results should be confirmed using an alternative assay method.

10.2 This assay has not been validated using paediatric samples.

10.3 Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to supplier.

10.4 Diagnosis cannot be made and treatment must not be given on the basis of microalbumin measurements alone. Clinical history and other laboratory findings must be taken into account.

10.5 Variation in reagent temperature may affect results. Ensure that reagents are transferred directly from the refrigerator to the refrigerated reagent compartment of the analyser – do not allow to warm to room temperature.

11 EXPECTED VALUES

The ranges provided have been obtained from a limited number of adult samples and are intended for guidance purposes only. Wherever possible it is strongly recommended that local ranges are generated.

Adult urine ranges

The following reference interval applies to urine samples from healthy adults:

Microalbumin <30.0 mg/L

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A study was performed following CLSI *Evaluation of Precision Performance of Clinical Quantitative Measurement Methods; Approved Guideline* (CLSI Document EP5-A2)**.** The study was performed over 21 working days, with two runs per day. One user assessed three different samples using three different reagent lots on three analysers.

Microalbumin Precision summary									
	Mean	Within run		Between run		Between day		Total	
	(mg/L)	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Urine 1	281.57	6.05	2.1	4.55	1.6	21.83	7.8	23.11	8.2
Urine 2	144.18	2.69	1.9	1.38	1.0	9.74	6.8	10.19	7.1
Urine 3	20.14	0.34	1.7	0.47	2.3	1.08	5.4	1.22	6.1

12.2 Comparison

A correlation study was performed on 79 samples (2 normal urines and 77 clinical urines) using this kit on a SPAPLUS and an alternative commercially available Microalbumin assay. The study demonstrated excellent agreement with the following Passing Bablok plot:

y = 0.99x+ 0.14 (mg/L)

(y = SPAPLUS Microalbumin; x = alternative assay)

correlation coefficient r = 1.00 (calculated by linear regression)

12.3 Limit of Quantitation

Based on *CLSI document EP17-A - Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline* the limit of quantitation for this assay is defined as the lowest point of the calibration curve i.e. 10.193mg/L based upon measuring neat sample.

12.4 Linearity

A linearity study was performed following CLSI (formerly NCCLS) *Evaluation of the Linearity of Quantitative Measurement Procedures* document EP6-A. One user assessed the linearity of a pool of high samples using one lot of reagent on one analyser. This gave a regression plot of y = 1.012x – 3.818 (y = measured Microalbumin concentration, x = theoretical concentration) over the range of 8.745 – 408.53mg/L using the analyser's 1/1 sample dilution.

12.5 Interference

No significant assay interference by 200mg/L ascorbic acid, 200mg/L bilirubin, 250mg/L haemoglobin and 1000mg/L total protein has been demonstrated at the minimum sample dilution (1/1).

	Ascorbic acid	Bilirubin	Hb	Total protein
Mean microalbumin (mg/L)	35.24	31.75	35.03	34.56
% interference	-1.38	-3.00	0.11	1.10

12.6 Antigen excess

No antigen excess was observed to a level of 8.0g/L.

13 BIBLIOGRAPHY

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.