Human α2-Macroglobulin Kit for use on SPAPLUS®

For *in-vitro* diagnostic use

Product Code: NK039.S

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FDA (USA) Information:
Analyte Name: Alpha-2-macroglobulin
Complexity Category: Moderate

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1 INTENDED USE

This kit is designed for the quantitative *in vitro* measurement of alpha 2-Macroglobulin in human serum using the SPAPLUS turbidimetric analyser. Measurement of alpha 2-Macroglobulin may aid in the diagnosis of blood-clotting or clot lysis disorders. This test should be used in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

Alpha 2-Macroglobulin is a 725 kDa protein and, because of its high molecular weight, it is distributed almost exclusively in the intravascular pool (Ref 1). The levels of alpha 2-Macroglobulin are reported to increase in the nephrotic syndrome (Ref 2), liver cirrhosis and diabetes mellitus where lower molecular weight proteins are leaked from the kidneys into the urine (Ref 3).

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 α2-Macroglobulin antiserum:** This antiserum is monospecific for α2-Macroglobulin 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 α2-Macroglobulin 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 is for *in vitro* diagnostic prescription use only and 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 α2-Macroglobulin 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 α2-Macroglobulin antiserum and reaction buffer may be stored, uncapped, on the SPAPLUS 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 and the temperature remains between 8-12°C.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen serum samples.
Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 2-8°C for up to 7 days prior to assay, or for prolonged storage kept at -20°C or below (Ref 4). Repeated freezing and thawing should be avoided. Microbially contaminated, haemolysed and lipaemic serum and samples containing particulate matter should not be used.

8 METHODOLOGY

8.1 Materials Provided

8.1.1 1 x 100 Tests *Human α2-Macroglobulin Antiserum SPAPLUS*
8.1.2 1 x *Human α2-Macroglobulin SPAPLUS Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1.5mL *Human α2-Macroglobulin SPAPLUS High Control*
8.1.4 2 x 1.5mL *Human α2-Macroglobulin SPAPLUS Low Control*
8.1.5 1 x 100 Tests *α2-Macroglobulin Reaction Buffer SPAPLUS*

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 SPAPLUS analyser.
8.2.3 Current analyser operating instructions: SPAPLUS Reference Guide, Insert Code FIN012.
8.2.4 Sample Diluent (99: Dil 1) Product Code: SN080.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 SPAPLUS 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 SPAPLUS Reference Guide (FIN012) supplied with the analyser.

8.4.1 Test parameters

8.4.1.1 **Assay parameters are entered into item number 41**

Item Name 41 A2M
DATA INFORMATION
Units g/L
Decimals 3

ANALYSIS
Type End▼

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

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION
Type Logit 2 ▼
Auto fill
Standard
1 # 4 #
2 # 5 #
3 # 6 #

NORMAL RANGE

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

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Item Name 41 A2M
ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 20
REAGENT1 VOL 200 µL
REAGENT2 VOL 40

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

MONITOR
0 LEVEL SPAN 1
SPAN 3.0

DATA PROCESS
READ

	START	END
MAIN	53	54
SUB	30	31

ABSORBANCE LIMIT
LOW -3.0
HIGH 3.0

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 10 ▼
Auto Rerun Dilution Rate High ▼
Auto Rerun Dilution Rate Low ▼

PROZONE CHECK
0

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

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*Automatically calculated

Item Name 41 A2M
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	21.0	Reagent2 R1	0
Reagent2 R2	5.0	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 (SIN241.QC). Calibrator values on **Page 1** should be entered in ascending order, i.e. lowest value first. The analyser will automatically calculate and enter the correct measuring ranges on Item pages 3 and 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 Measuring range

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

SPAPLUS Analyser Dilution	Approximate range (g/L)
1/10	0.2 – 6.4

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 regular 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 (SIN241.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 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.

10.2 This assay has not been validated using paediatric samples, samples taken from pregnant females or individuals being administered oestrogen.

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 α2-Macroglobulin 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 samples and are intended for guidance purposes only. Wherever possible it is strongly recommended that local ranges are generated.

Adult serum range

The reference range was established by measuring the α2-Macroglobulin concentration of sera taken from 166 adult donors. The study was based on CLSI document *EP C28-A3 "Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory"*.

	Number (n)	95 Percentile Range (g/L)
Human α2-Macroglobulin	166	0.74 – 2.98

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A study was based on 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.

Level	Mean (g/L)	Within Run		Between run		Between day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.34	0.005	1.6	0.014	4.1	0.045	13.3	0.047	14.0
2	2.29	0.024	1.0	0.049	2.1	0.092	4.0	0.107	4.7
3	3.45	0.034	1.0	0.076	2.2	0.149	4.3	0.171	4.9
4	4.19	0.055	1.3	0.099	2.4	0.178	4.3	0.211	5.0
5	5.42	0.086	1.6	0.131	2.4	0.259	4.8	0.303	5.6

Level	Mean (g/L)	Between instrument		Between lot	
		SD	%CV	SD	%CV
1	0.34	0.017	4.9	0.040	11.8
2	2.29	0.076	3.3	0.048	2.1
3	3.45	0.107	3.1	0.031	0.9
4	4.19	0.121	2.9	0.095	2.3
5	5.42	0.187	3.5	0.161	3.0

12.2 Comparison

A correlation study was performed on 154 samples using this kit on a SPAPLUS and an alternative commercially available α2-Macroglobulin assay. The study demonstrated excellent agreement with the following Passing Bablok plot:

y = 1.00x - 0.05 (g/L) (y = SPAPLUS α2-Macroglobulin; x = alternative assay)

correlation coefficient r = 0.977 (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 detection represents the lowest measurable analyte level that can be distinguished from zero, and has been established as 0.03 g/L.

The limit of quantitation for this assay is defined as the lowest point of the calibration curve i.e. 0.20 g/L based upon the minimum sample dilution.

12.4 Linearity

A linearity study based on CLSI (formerly NCCLS) *Evaluation of the Linearity of Quantitative Measurement Procedures* document EP6-A was performed. One user assessed the linearity of a pool of high samples using one lot of reagent on one analyser. This gave a linear regression plot of y = 1.01x - 0.06 (y = measured α2-Macroglobulin concentration, x = theoretical concentration) over the measuring range of the assay using the analyser's 1/10 sample dilution.

12.5 Interference

A study was performed based on CLSI EP7-A2: *Interference Testing in Clinical Chemistry, Approved Guideline*. A normal serum sample, a serum sample close to the medical decision point and a clinical serum sample were tested. No significant assay interference effects were observed when tested with triglyceride (1000mg/dL), Intralipid (500mg/dL), bilirubin (200mg/L) or haemoglobin (5g/L).

12.6 Antigen excess

No antigen excess was observed to a level of twice the top point of the assay; approximately 12.8 g/L.

13 BIBLIOGRAPHY

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