Human α1-Acid Glycoprotein kit for use on the SPAPLUS®

For *in vitro* diagnostic use

Product Code: NK063.S

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

FDA (USA) Information
Analyte name: Alpha-1-Acid Glycoprotein (Orosomucoid)
Complexity Cat.: Moderate

CE

1 INTENDED USE

This kit is designed for the quantitative *in vitro* determination of α1-acid glycoprotein (AGP) in human serum using the SPAPLUS turbidimetric analyser. This test should be used in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

AGP also known as orosomucoid is a 41-43 kDA glycoprotein. It is one of the major acute phase proteins and is an important protein in drug binding. Elevated levels of AGP are found during acute and chronic inflammatory processes and infections. Low levels of AGP are found when there is a reduction in synthesis, e.g. in chronic liver disease or increased excretion of AGP, e.g. in nephrotic syndrome (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 AGP antiserum:** This antiserum is monospecific for AGP 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 AGP 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 AGP 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 AGP 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.

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 5 months prior to assay, or for one year when kept at -20°C or below (ref. 3). 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 α1-Acid Glycoprotein Antiserum SPAPLUS*
8.1.2 1 x *Human α1-Acid Glycoprotein SPAPLUS Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1.5mL *Human α1-Acid Glycoprotein SPAPLUS High Control*
8.1.4 2 x 1.5mL *Human α1-Acid Glycoprotein SPAPLUS Low Control*
8.1.5 1 x 100 Tests *α1-Acid Glycoprotein 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

Assay parameters are entered into item number 42.

Item Name 42 AGP
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 Spline1 ▼ 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 42 AGP

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 10
REAGENT1 VOL 160 µL
REAGENT2 VOL 40

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

MONITOR
0 LEVEL SPAN 1
SPAN 3.0

DATA PROCESS
READ **ABSORBANCE LIMIT**

	START	END	
MAIN	53	54	LOW -3.0
SUB	30	31	HIGH 3.0

FACTOR **Reaction Check**
Blank correction * ○ ON ● OFF
ENDPOINT LIMIT 2.0 CHECK POINT
LINEAR CHECK (%) 0 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

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

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*Automatically calculated

Item Name 42 AGP

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		

Auto Rerun Condition (Absorbance)
Absorbance Range
Lower ○ On ● Off
Higher ○ On ● Off
Prozone Range ○ On ● Off

Bottle Size (ml)

24 Items		36 Items	
Reagent1	60	Reagent1	0
Reagent2 R1	17.0	Reagent2 R1	0
Reagent2 R2	5.0	Reagent2 R2	0

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The calibrator (Standard #) values are found in the Quality Control Certificate (SIN245.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 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.
* The Blank correction factor is automatically calculated by the instrument.

8.5 Measuring range

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

SPAPLUS Analyser Dilution	Approximate range (g/L)
1/10	0.19 – 6.0

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 (SIN245.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.

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 AGP 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 for this kit was transferred from an alternative commercially available assay in accordance with CLSI document *EP C28-A3 "Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory"* and was validated by measuring the AGP concentration of sera taken from 20 healthy UK adults. Wherever possible it is strongly recommended that local ranges are generated.

	Number (n)	95 Percentile Range (g/L)
AGP	20	0.43 – 1.07

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.

AGP precision summary									
	Mean (g/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	5.014	0.14	2.8	0.00	0.0	0.255	5.1	0.290	5.8
Serum 2	1.300	0.02	1.5	0.034	2.6	0.078	6.0	0.088	6.7
Serum 3	0.2923	0.005	1.8	0.005	1.5	0.031	10.6	0.032	10.8

12.2 Comparison

A correlation study was performed on 75 samples (using a variety of normal and clinical sera) using this kit on a SPAPLUS and an alternative commercially available AGP assay. The study demonstrated excellent agreement with the following Passing & Bablok fit:

y = 0.92x – 0.03 (g/L) (y = SPAPLUS AGP; x = alternative assay)

correlation coefficient r = 0.996 (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. 0.19g/L based upon a 1/10 sample dilution.

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 = 0.961x – 0.035 (y = measured AGP concentration, x = theoretical concentration) over the range of 0.153 – 7.167 g/L using the analyser's 1/10 sample dilution.

12.5 Interference

Interference by 1500 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, 5.0g/L haemoglobin has been determined to be below d_{max}, defined as the maximum level of interference considered acceptable (0.05g/L), at the standard sample dilution (1/10).

	Bilirubin	Hb	Chyle
d_{obs} (g/L)	0.011	-0.003	0.005
d_{obs}, 95% CI (g/L)	-0.024 – 0.046	-0.038 –0.032	-0.030 – 0.039

12.6 Antigen excess

No antigen excess was observed to a level of two times the top point of the assay; approximately 12.0g/L.

13 BIBLIOGRAPHY

1. T. Fournier, N. Medjoubi-N, D. Porquet (2000) Review Alpha-1-acid glycoprotein. Biochimica et Biophysica Acta 1482, 157 - 171
2. P.D Booker, C. Taylor, G Sana (1996) Perioperative changes in α1-Acid glycoprotein concentrations in infants undergoing major surgery 76, 365 -368
3. Use of Anticoagulants in Diagnostic Laboratory Investigations WHO/DIL/LAB/99.1 Rev.2 2002