

Human Prealbumin Kit for use on SPAPLUS®

For *in vitro* diagnostic use Product Code: NK066.S

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FDA (USA) Information
Analyte Name: Prealbumin
Complexity Cat.: Moderate



1 INTENDED USE

This kit is designed for the *in vitro* measurement of human prealbumin in serum using the Binding Site SPAPLUS turbidimetric analyser as an aid in the assessment of nutritional status. This test should be used in conjunction with other laboratory and clinical findings

2 SUMMARY AND EXPLANATION

Prealbumin is a serum protein that has a high affinity for and binds with both thyroxine and retinol binding protein. It is primarily synthesized in the liver, but is also produced by the choroid plexus. Prealbumin concentrations are dependent on protein and energy intake and reduced levels are associated with malnutrition (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

- Human prealbumin antiserum:** This antiserum is monospecific for prealbumin 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.
- Calibrator and controls:** These consist of pooled human serum and are supplied in stabilised liquid form. The concentration of prealbumin given on the quality control certificate has been obtained by comparison with the DA470k International Reference Material. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.
- 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 Prealbumin 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 Prealbumin 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 7 days prior to assay. For prolonged storage freeze within 24 hours after collection and store for up to 3 months at -20°C or below. 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

- 1 x 100 Tests Human Prealbumin Antiserum SPAPLUS
- 1 x Human Prealbumin SPAPLUS Calibrator set 1-6 (6 x 1.0mL)
- 2 x 1.5mL Human Prealbumin SPAPLUS High Control
- 2 x 1.5mL Human Prealbumin SPAPLUS Low Control
- 1 x 100 Tests Prealbumin Reaction Buffer SPAPLUS

8.2 Materials required but not provided

- Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
- A fully operational and equipped SPAPLUS analyser.
- Current analyser operating instructions: SPAPLUS Reference Guide, Insert Code FIN012.
- 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 35.

Item Name 35 PALB		CALIBRATION		Auto Fill	
DATA INFORMATION		Type	Logit 2		
Units	g/L	Standard			
Decimals	3	1 #	4 #		
ANALYSIS		2 #	5 #		
Type	End	3 #	6 #		
Main W.Length 1	340				
Sub W.Length					
Method					
CORR.		NORMAL RANGE			
Y =	SLOPE INTER	Serum	LOW HIGH	MALE	FEMALE
1	X + 0	Urine	[] []	LOW HIGH	[] []
		Plasma	[] []	[] []	[] []
		CSF	[] []	[] []	[] []
		Dialysis	[] []	[] []	[] []
		Other	[] []	[] []	[] []
Page : 1		Print	Hard Copy	Next Page	Save Return

Item Name 35 PALB		DATA PROCESS		ABSORBANCE LIMIT	
ASPIRATION		READ	START END		
KIND	Single Double	MAIN	53 54	LOW	3.0
VOLUME	20	SUB	30 31	HIGH	3.0
SAMPLE					
REAGENT1 VOL	200 µL				
REAGENT2 VOL	20				
Third mix		FACTOR			
R1 Blank	Water - Blank	Blank correction	*	Reaction Check	
		ENDPOINT LIMIT	2.0	ON OFF	
		LINEAR CHECK (%)	0	CHECK POINT	
				LOW -3	
				HIGH 3	
MONITOR		DILUTION			
0 LEVEL SPAN 1		Diluent	99: Dil 1	100: Dil 2	
SPAN 3.0		Pre Dilution Rate	10		
		Auto Reun Dilution Rate High			
		Auto Reun Dilution Rate Low			
PROZONE CHECK		START END LIMIT (%) Min dOD			
FIRST	[] [] [] []				
SECOND	[] [] [] []				
THIRD	[] [] [] []				
Page : 2		Print	Hard Copy	Prev Page	Next Page Save Return

*Automatically calculated

Item Name 35 PALB		Auto Reun SW		Auto Reun Condition (Absorbance)	
Auto Reun Range (Result)		Lower Higher		Absorbance Range	
On Off On Off		On Off		Lower On Off	
On Off On Off		On Off		Higher On Off	
Serum Cal 1# Cal 6#		Prozone Range		On Off	
Urine					
Plasma					
CSF					
Dialysis					
Other					
Bottle Size (ml)					
24 Items	36 Items				
Reagent1 60.0	Reagent1				
Reagent2 R1 21.0	Reagent2 R1				
Reagent2 R2 3.0	Reagent2 R2				
Page : 3		Print	Prev Page	Save	Return

The calibrator (Standard #) values are found in the Quality Control Certificate (SIN211.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.

8.5 Measuring range

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

SPAPLUS analyser dilution	Approximate range (g/L)
1/1 (neat)	0.006 – 0.08
1/10	0.06 – 0.8

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 (SIN211.QC). Sample results obtained should only be accepted if the control results are within $\pm 15\%$ 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 prealbumin 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 each facility should determine its own reference intervals since values may vary depending on the individual population studied.

Adult serum ranges

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

Prealbumin (Ref. 3) 0.2 – 0.4 g/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 two different samples using three different reagent lots on three analysers.

	Prealbumin precision summary								
	Mean (g/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	0.474	0.006	1.2	0.006	1.2	0.013	2.6	0.015	3.1
Serum 2	0.108	0.002	1.7	0.002	1.6	0.006	5.2	0.006	5.7

12.2 Comparison

A correlation study was performed on 78 samples (a variety of normal and clinical sera) using this kit on a SPAPLUS and an alternative commercially available prealbumin assay. The study demonstrated excellent agreement with the following Passing Bablok plot:

$$y = 0.96x - 0.00 \text{ (g/L)} \quad (y = \text{SPAPLUS Prealbumin}; x = \text{alternative assay})$$

$$\text{correlation coefficient } r = 0.990 \quad (\text{calculated by linear regression})$$

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. 0.006g/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 $y = 0.95x + 0.01$ (y = measured prealbumin concentration, x = theoretical concentration) over the range of 0.075-0.934g/L using the analyser's 1/10 sample dilutions.

12.5 Interference

No significant assay interference by 1500 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin or 5.0g/L haemoglobin has been demonstrated at the minimum sample dilution (1/1).

	Bilirubin	Hb	Chyle
Mean Prealbumin (g/L)	0.023	0.022	0.035
% interference	-2.82%	-2.94%	-8.70%

12.6 Antigen excess

No antigen excess was observed to a level of 7.3 times the top point of the assay; approximately 4.75g/L.

13 BIBLIOGRAPHY

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- Dati F, Schumann G, Thomas L, *et al.* Consensus of a Group of Professional Societies and Diagnostic Companies on Guidelines for Interim Reference Ranges for 14 Proteins in Serum Based on the Standardization against IFCC/BCR/CAP Reference Material (CRM470). [International Federation of Clinical Chemistry; Community Bureau of Reference of the Commission of the European Communities; College of American Pathologists]. Eur J Clin Chem Clin Biochem 1996; 34:517-520