Human C4 Kit
for use on SPAPLUS®

For *in vitro* diagnostic use only

Product Code: NK025.S

Product manufactured by:
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FDA (USA) Information
Analyte name Complement C4
Complexity Cat.: Moderate

CE

1 INTENDED USE

This kit is intended for the quantitative *in vitro* determination of human C4 in serum using the Binding Site SPAPLUS turbidimetric analyser. This test should be used in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

C4 is a component of the complement system, and has a fundamental role in the inflammatory response and immune system functionality. C4 is produced in the liver, and accordingly reduced complement activity is associated with severe liver failure (Ref, 1). Deficiencies in C4 manifest in recurrent staphylococcal and streptococcal infections, and immune complex disorders such as glomerulonephritis, vasculitis, and endocarditis (Ref, 2). Inherited or acquired deficiencies in C1q inhibitor cause decreased serum levels of C4, which are associated with angioedema (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 C4 Antiserum:** This is supplied in stabilised liquid form. Preservatives: 0.099% sodium azide, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine.

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 C4 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 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 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 C4 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 C4 Antiserum and Reaction Buffer may be stored at 8-12°C 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 serum samples. Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. Sera may be stored at 2-8°C for up to three days, otherwise aliquot and freeze at -20°C or below and store for up to 3 months; do not freeze and thaw sera more than once. Sample dilutions should be freshly prepared on the day of assay. **Note:** Upon storage, C4 degrades. Depending on the storage conditions, the C4 amounts in fresh samples are higher than in aged samples.

8 METHODOLOGY

8.1 Materials Provided

8.1.1 1 x 100 Tests *Human C4 Antiserum SPAPLUS*
8.1.2 1 x *Human C4 SPAPLUS Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1.3mL *Human C4 SPAPLUS High Control*
8.1.4 2 x 1.3mL *Human C4 SPAPLUS Low Control*
8.1.5 1 x 100 Tests *C4 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).

8.4.1 Test parameters

Assay parameters are entered into item number 20.

Item Name 20 C4
DATA INFORMATION
Units g/L
Decimals 3

ANALYSIS
Type End ▼

Main W.Length 1 380 ▼
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	[ ]	[ ]	[ ]	[ ]

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Item Name 20 C4

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 19.5
REAGENT1 VOL 165 µL
REAGENT2 VOL 20

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 20 ▼
Auto Rerun Dilution Rate Low ▼

PROZONE CHECK

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

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*Automatically calculated

Item Name 20 C4

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
Reagent2 R1	17.5	Reagent2 R1
Reagent2 R2	3	Reagent2 R2

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The calibrator (Standard #) values are found in the Quality Control Certificate (SIN168.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 the C4 assay when using the standard 1/10 sample dilution is 0.064-0.9g/L.

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 (SIN168.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 e.g. radial immunodiffusion.

10.2 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.

10.3 Diagnosis cannot be made and treatment must not be given on the basis of C4 measurements alone. Clinical history and other laboratory findings must be taken into account.

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. Paediatric data is not available.

Adult serum ranges

These ranges were obtained using this kit, by measuring the C4 concentration of sera taken from healthy adult UK blood donors. The reference interval was calculated using non-parametric statistics and represents the central 95% of the population.

	Number (n)	Mean (g/L)	Median (g/L)	95 Percentile Range (g/L)
C4	120	0.241	0.234	0.129-0.392

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.

C4 Precision Summary									
	Mean (g/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	0.738	0.014	1.9	0.026	3.6	0.018	2.4	0.035	4.7
Serum 2	0.167	0.004	2.1	0.005	2.8	0.012	6.9	0.013	7.8
Serum 3	0.111	0.002	1.9	0.005	4.6	0.006	5.6	0.008	7.5

12.2 Comparison

A correlation study was performed on 101 samples (31 normal serum and 70 clinical serum) using this kit on a SPAPLUS and an alternative commercially available C4 assay. The study demonstrated excellent agreement with the following Passing Bablok plot:

y = 0.99x + 0.02 (g/L) (y = SPAPLUS C4; x = alternative assay)

correlation coefficient r = 0.996 (calculated by linear regression)

12.3 Limit of Blank and Limit of Detection

Based on *CLSI document EP17-A - Protocols for Determination of Limits of Detection and Limits Quantitation; Approved Guideline* the limit of detection represents the lowest measurable analyte level that can be distinguished from zero. This has been estimated at 0.003g/L (n = 60).
The limit of quantitation for this assay is defined as the lowest point of the calibration curve i.e. 0.06g/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 diluted high level pool of samples using one lot of reagent on one analyser. This gave a regression plot of y = 1.005x -0.014g/L (y = measured C4 concentration, x = theoretical concentration) over the range of 0.06-1.14g/L.

12.5 Interference

No significant assay interference by 1500 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, 5g/L haemoglobin has been demonstrated at the minimum sample dilution (1/1).

	Bilirubin	Hb	Chyle
Mean C4 (g/L)	0.048	0.051	0.049
% interference	-5.3%	4.8%	-2.0%

12.6 Antigen excess

No antigen excess was observed to a level of four times the top point of the assay; approximately 5.5g/L.

13 BIBLIOGRAPHY

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3. Milford-Ward, E. A. Riches, P. G. Fifield, R. Smith, A. M. (1999) Protein Reference Unit Handbook of Clinical Immunochemistry. Publ PRU Publications, Sheffield, UK. p76-77.