Human CYSTATIN C Kit for use on the SPAPLUS®

For *in vitro* diagnostic use only

Product code: LK048.S

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FDA (USA) Information

Analyte name	Cystatin C
Complexity Cat.	Moderate

CE

1 INTENDED USE

This cystatin C kit is intended for the quantitative determination of cystatin C in human serum, lithium heparin plasma and EDTA plasma by turbidimetry using the SPAPLUS analyser. Cystatin C measurements in serum and plasma are used as an aid in the diagnosis and treatment of renal diseases in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

Cystatin C is a 13kDa, non-glycosylated endogenous cysteine protease inhibitor that is produced by all nucleated cells at a constant rate. It is freely filtered by the glomerulus, and is almost completely reabsorbed and degraded by the proximal tubular cells (ref 1). The production rate of cystatin C is not affected by age, gender, muscle mass or inflammatory processes, making serum cystatin C an ideal marker of glomerular filtration rate (GFR). A reduction in the GFR results in an increase in cystatin C concentrations. Cystatin C has been shown to be superior to creatinine as marker for GFR in several studies and a meta-analysis (ref 2-7).

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.

Latex-enhanced antibodies Some antigen-antibody reactions do not form sufficiently large immune complexes to be detected turbidimetrically. If the antibody is coated onto latex particles of a suitable size, the light scattering ability of the immune complexes formed with antigen is enhanced sufficiently to enable turbidimetric detection.

4 REAGENTS

4.1 **Latex reagent:** Consisting of monospecific sheep antibody coated onto polystyrene latex. Preservative: 0.05% Proclin™*, 0.025% sodium azide, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine.

4.2 **Calibrators and controls:** These consist of pooled human serum and are supplied in stabilised liquid form. . The concentration of Cystatin C given on the quality control certificate has been obtained by comparison with ERM-DA471/IFCC European Reference Material. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.

4.3 **Supplementary reagent:** Containing 0.099% sodium azide as a preservative.

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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 ProClin 300 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 reagents, 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 Cystatin C Reagent and Cystatin C Supplementary Reagent 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, lithium heparinised or EDTA plasma samples. Samples should be obtained by venepuncture and in the case of plasma separated as soon as possible. Blood should be allowed to clot and the serum separated as soon as possible to prevent haemolysis. Samples may be stored at 2-8°C for up to 7 days, but for prolonged storage samples should be kept frozen at -20°C or below. Repeated freeze/thaw cycles should be avoided. Microbially contaminated serum samples, samples containing particulate matter and lipaemic or haemolysed serum samples should not be used.

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 100 Tests *Human Cystatin C Reagent*
8.1.2 1 x *Human Cystatin C SPAPLUS Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1.0mL *Human Cystatin C High Control*
8.1.4 2 x 1.0mL *Human Cystatin C Low Control*
8.1.5 1 x 100 Tests *Cystatin C Supplementary Reagent*

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 3.

Item Name 3 CysC
DATA INFORMATION
Units mg/L
Decimals 2

ANALYSIS
Type End▼

Main W.Length 1 600 ▼
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 3 CysC

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 4
REAGENT1 VOL 160 µL
REAGENT2 VOL 80

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 35 36

ABSORBANCE LIMIT
LOW -3.0
HIGH 3.0

FACTOR
Blank correction 1
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	0
FIRST	[ ]	[ ]			
SECOND	[ ]	[ ]	[ ]	○ Low ● High	
THIRD	[ ]	[ ]	[ ]	○ Low ● High	

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Item Name 3 CysC

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	32.5
Reagent2 R1	17	Reagent2 R1	26.25
Reagent2 R2	9	Reagent2 R2	13

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 (SIN126.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 measuring range of the Cystatin C assay when using the standard 1/10 sample dilution is 0.4-7.35mg/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 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 (SIN126.QC). Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.

10 LIMITATIONS

10.1 Nephelometric or 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 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 cystatin C 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 samples and are intended for guidance purposes only. Wherever possible it is strongly recommended that local ranges are generated.

Adult serum ranges

Revised September 2010 following calibration to ERM-DA471/IFCC.
These ranges were obtained using this kit, by measuring the cystatin C 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 (mg/L)	Median (mg/L)	95 Percentile Range (mg/L)
Cystatin C	127	0.72	0.70	0.56-0.99

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A study was performed following NCCLS *Evaluation of Precision Performance of Clinical Chemistry Approved Guideline* (NCCLS Document EP5-A)**.** 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.

Cystatin C Precision Summary									
	Mean	Within run		Between run		Between day		Total	
	(mg/L)	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	6.029	0.11	1.8	0.11	1.8	0.29	4.7	0.32	5.4
Serum 2	0.963	0.02	1.9	0.02	2.4	0.05	5.4	0.06	6.2
Serum 3	0.533	0.02	2.8	0.01	1.9	0.05	8.8	0.05	9.4

12.2 Comparison

A correlation study was performed on 68 serum samples (30 normal, 38 from known renal impaired patients) and 53 plasma samples (20 normal, 33 from known renal impaired patients) using this kit and an alternative commercial cystatin C assay on the BNII. The study demonstrated excellent agreement with the following Deming regression plot:

y= 1.02x + 0.07 (mg/L) (y = Binding Site Cystatin C; x = alternative assay)

correlation coefficient r = 0.995 (calculated by linear regression)

12.3 Analytical sensitivity

Analytical sensitivity was determined by assaying ten replicates of two samples with concentrations equivalent to 140% (0.05mg/L) and 200% (0.07mg/L) of the curve bottom point. Two distinct sets of data were generated with CV's of 1.6% and 0.9% respectively.

12.4 Linearity

The linearity of this assay has been confirmed using serially diluted serum samples, giving a regression equation of *y=1.0015x - 0.009 (mg/L), r =0.999.* (y = measured cystatin C concentration, x = theoretical concentration) over the range of 0.05-7.24mg/L.

12.5 Interference

Minimal assay interference by 200mg/L bilirubin (-4.4%), 5g/L haemoglobin (+8.2%) and 0.5% intralipid (-2.2%) has been demonstrated using a sample containing 0.45mg/L cystatin C. Rheumatoid factor (320IU/mL) showed no significant interference (+0.33%) when added to a serum sample containing 0.61mg/L of cystatin C.

12.6 Antigen excess

No antigen excess was observed to a level of approximately 17mg/L.

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