Full Range C-Reactive Protein Kit for use on SPAPLUS®

For *in vitro* diagnostic use

Product code: LK044.S

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1 INTENDED USE

The Full Range C-Reactive Protein Kit for use on SPAPLUS is intended for the quantitative *in vitro* determination of C-reactive protein (CRP) concentration in serum or plasma. This product is suitable for use on the SPAPLUS analyser.

2 SUMMARY AND EXPLANATION

C-reactive protein is present in serum of normal individuals at levels from 0-5mg/L. Elevated levels outside the normal range are associated with acute phase response and measurements may be useful for the detection of infection, tissue injury, inflammatory disorders and associated diseases.
Research has indicated that CRP levels within the normal range can be used in a variety of different population subgroups for the assessment of cardiovascular risk for developing a fatal myocardial infarction. CRP levels within the normal range have been associated with coronary heart disease mortality in high-risk individuals. Elevated serum cholesterol levels, elevated diastolic blood pressure and cigarette smoking are associated with high-risk.
A complete clinical history is required for accurate interpretation of CRP levels. CRP levels within the normal range may be affected by a number of different factors and should always be compared to previous values.[5,6]

3 PRINCIPLE

Sample is reacted with anti-CRP coated latex in reaction buffer. The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, the extent of which is measured as the amount of light absorbed at 570nm. By constructing a standard curve from the absorbance of the standards, CRP concentration of sample can be determined.

4 REAGENTS

4.1 R1. frCRP Reaction Buffer: Containing Glycine buffer 170mM, sodium chloride 100mM, sodium EDTA disodium salt dihydrate 50mM, bovine serum albumin 1% (w/v).
4.2 R2. frCRP Reagent: Latex particles coated with antibody to CRP.
4.3 frCRP Controls: Supplied at 4 levels, Ultra Low, Low, Medium, and Elevated. Target values and ranges are supplied in the Quality Control certificate. Supplied ready for use.
4.4 frCRP Calibrator 0-5: Calibration has been carried out and values have been assigned using an immunoturbidimetric method with reference material standardised against CRM470. Supplied ready for use.

5 CAUTION

Reagent: For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.
Solutions R1 and R2 contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
Health and Safety data sheets are available on request.

Calibrator and Controls: For *in vitro* diagnostic use only. Human source material from which this product has been derived has been tested at donor level for the Human Immunodeficiency Virus (HIV 1, HIV 2) antibody, Hepatitis B Surface Antigen (HbsAg), and Hepatitis C Virus (HCV) antibody and found to be NON-REACTIVE. FDA cleared methods have been used to conduct these tests. However, since no method can offer complete assurance as to the absence of infectious agents, this material and all patient samples should be handled as though capable of transmitting infectious diseases and disposed of accordingly.

6 STORAGE AND STABILITY

R1. frCRP Reaction Buffer
Supplied ready for use. Stable up to expiry date when stored at 2-8°C. The *frCRP Reaction Buffer* may be stored, uncapped, on the 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.
R2. frCRP Reagent
Supplied ready for use. Stable up to expiry date when stored at 2-8°C. Invert several times before use, avoiding the formation of foam. The *frCRP Reagent* may be stored, uncapped, on the 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.
frCRP Controls
OPENED: Store refrigerated (2-8°C). frCRP control material is stable for 30 days at 2-8°C if kept capped in original container and free from contamination. Only the required amount of product should be removed. After use, any residual product should NOT BE RETURNED to the original vial.
UNOPENED: Store refrigerated (2-8°C). Stable to expiration date printed on individual vials.
frCRP Calibrator 0-5
The material is stable up to expiry when stored at 2-8°C in the original capped containers. Open vials are stable for 30 days in the absence of bacterial contamination. Only the required amount of product should be removed. After use, any residual product should NOT BE RETURNED to the original vial.

7 SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (K-EDTA or Li-Heparin). Fresh serum is recommended. Stable for 11 days at 15-25°C or 2 months at 2-8°C. Sample can be frozen once at -15 to -25°C for a maximum of 3 years (do not refreeze).[7]

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 100 tests *frCRP Reagent SPAPLUS*.
8.1.2 1 x 100 tests *frCRP Reaction Buffer SPAPLUS*.
8.1.3 1 x *frCRP SPAPLUS Calibrator 0-5* (6 x 1.0mL calibrator set for CRP).
8.1.4 1 x 1.0mL *CRP SPAPLUS Ultra Low Control*
8.1.5 1 x 1.0mL *CRP SPAPLUS Low Control*
8.1.6 1 x 1.0mL *CRP SPAPLUS Medium Control*
8.1.7 1 x 1.0mL *CRP SPAPLUS Elevated Control*

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). Binding Site 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.

Note: Zero calibrator is used for the blank reading on this assay. Transfer 150µL of Calibrator 0 into a sample cup and place into position B-1 or B-2 of the Calibration rack when running calibration.

8.4.1 Test parameters

Assay parameters are entered into item number 29.

Item Name 29 CRP

DATA INFORMATION
Units mg/L
Decimals 2

ANALYSIS
Type End ▼

Main W.Length 1 570 ▼
Sub W.Length 800 ▼

Method

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION
Type Spline 1 ▼ 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	[ ]	[ ]	[ ]	[ ]

Page : 1 Print Hard Copy Next Page Save Return

Item Name 29 CRP

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 4
REAGENT1 VOL 100 µL
REAGENT2 VOL 100

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

DATA PROCESS
READ **ABSORBANCE LIMIT**
START END
MAIN 49 50 LOW -3
SUB 35 36 HIGH 3

FACTOR **Reaction Check**
Blank correction * ○ ON ● OFF
ENDPOINT LIMIT 2 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 ▼

MONITOR

0 LEVEL SPAN 1
SPAN 3

PROZONE CHECK

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

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*Automatically calculated

Item Name 29 CRP

Auto Rerun SW
● On ○ Off

Auto Rerun Range (Result)
● On ○ Off ○On ● Off

	Lower	Higher
Serum	#	#
Urine	#	#
Plasma	#	#
CSF	#	#
Dialysis	#	#
Other	#	#

Bottle Size (ml)

24 Items		36 Items	
Reagent1	60	Reagent 1	0
Reagent2 R1	11	Reagent2 R1	0
Reagent2 R2	11	Reagent2 R2	0

Auto Rerun Condition (Absorbance)

Absorbance Range
Lower ○ On ● Off
Higher ● On ○ Off

Prozone Range ○ On ● Off

Page : 3 Print Prev Page Save Return

Item Name 29 CRP

Out-of-Range Table

	NEAT		Pre Dilution (*10)		Auto-rerun Dilution	
	ABOVE Cal#1	BELOW Cal#2-Cal#6	ABOVE Cal#1	BELOW Cal#2-Cal#6	ABOVE Cal#1	BELOW Cal#2-Cal#6
Serum	#	#	#	#	#	#
Urine	#	#	#	#	#	#
Plasma	#	#	#	#	#	#
CSF	#	#	#	#	#	#
Dialysis	#	#	#	#	#	#
Other	#	#	#	#	#	#

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N.B. The calibrator (Standard #) values are found in the Quality Control certificate (SIN217.QC). Calibrator values on **Page 1** should be entered in ascending order, i.e. the lowest value first. IMPORTANT: the analyser will only update the calibrator values providing the Auto Fill button is pressed after typing the value for calibrator 5 on Page: 1.The assay utilises an extrapolated calibration curve therefore the Auto Rerun Range (Result) (Page 3) and Out-of-Range (Page 4) tables must be manually updated using values supplied in the Quality Control certificate (SIN217.QC)

8.4 Measuring range

The range of this assay is approximately 0.2 – 400 mg/L. These values are dependent on the lot specific value of the calibrator in use.

SPAPLUS analyser dilution	Approximate measuring range (mg/L)
1/1	0.2 – 40
1/10	10 - 400

8.5.1 hs-CRP Application

Samples and controls that are expected to return results of <10mg/L (high sensitivity-CRP) should be run with a starting dilution of 1/1 to minimise reagent usage.

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 (SIN217.QC). Sample results obtained should only be accepted if the control results are within ±20% of the concentration(s) stated.

10 LIMITATIONS

10.1 These kits are not suitable for the measurement of samples containing circulating immune complexes (CICs) or for lipaemic or haemolysed samples 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 Diagnosis cannot be made and treatment must not be initiated on the basis of CRP measurements alone. Clinical history and other laboratory findings must also be taken into account.

10.3 Antigen excess is a rare event but cannot be excluded. If the CRP results do not agree with other clinical or laboratory findings, or if the sample is from a patient that has previously demonstrated antigen excess, the result must be checked by retesting at a higher dilution. Results should always be interpreted in conjunction with other laboratory tests and clinical evidence.

11 EXPECTED VALUES

Age	Range
Adults	0 - 5 mg/L
Newborns, cord blood	<0.6 mg/L
Infants from 4th day of life to 1 month	<1.6 mg/L

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

frCRP precision summary						
	n	Mean (mg/L)	Intra assay		Total precision	
			SD	CV %	SD	CV %
Serum 1	80	1.17	0.03	2.5%	0.06	5.0%
Serum 2	80	4.31	0.06	1.4%	0.16	3.7%
Serum 3	80	137.86	1.47	1.1%	4.26	3.1%

12.2 Comparison

This method (Y) was compared with another commercially available method (X) and the following Passing & Bablok equation obtained:

$$y = 0.97x + 0.07$$

and a correlation coefficient of r = 0.999

99 patient samples were analysed spanning the range 0.24 to 148.6 mg/L.

12.3 Analytical sensitivity

The limit of Quantitation (LoQ), the limit of Detection (LoD) and the limit of Blank (LoB) were determined consistent with CLSI guidelines EP17-A2. LoQ is the smallest concentration that can be detected reliably. LoD is the smallest concentration that can be detected to determine the presence or absence of CRP. LoB is the highest concentration that is likely to be observed in a blank sample.

SPAPLUS

	CRP concentration mg/L
Limit of Blank	0.00
Limit of Detection	0.046
Limit of Quantitation	0.2

12.4 Linearity

The method is linear up to a CRP concentration of 40 mg/L at the 1/1 dilution.
This gave a regression plot of y = 1.06 x - 1.60; r = 0.999 (y = measured CRP concentration, x = theoretical concentration).

12.5 Interference

The analytes below were tested up to the following levels and were found not to interfere:

Haemoglobin 1000 mg/dL
Intralipid 2000mg/dL
Free Bilirubin 60mg/dL
Conjugated Bilirubin 60mg/dL
Triglycerides 2000mg/dL

12.6 Antigen excess

Antigen excess effects are not noted until levels approach 500 mg/L.

13 BIBLIOGRAPHY

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