Hevylite® Human IgG Kappa Kit for use on the SPAPLUS®

For *in vitro* diagnostic use

Product code: NK621.S

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CE

1 INTENDED USE

This kit is intended for the *in vitro* quantification of IgG kappa in human serum on the Binding Site SPAPLUS.

2 SUMMARY AND EXPLANATION

Immunoglobulins are produced after the exposure of the humoral immune system to specific antigens. IgM is the first class of immunoglobulin produced. With maturation of the response, IgG and IgA antibodies may also be produced. Immunoglobulin molecules consist of two identical heavy chains (α, δ, ε, γ or μ) which define the immunoglobulin class and two identical light chains (κ or λ). Each light chain is linked to a heavy chain and the two heavy chains are linked covalently at the hinge region. In healthy individuals, the IgG concentration ranges from 6.0 - 16.0g/L[1].
Elevated serum concentrations of monoclonal protein are indicative of an underlying abnormality such as monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma and other lymphoproliferative disorders. International guidelines[2] recommend serum protein electrophoresis (SPE) densitometry is performed to quantify monoclonal proteins. Turbidimetry can also be used in these instances to measure total IgG but this will include non-tumour immunoglobulin, and measurement of either IgGκ or IgGλ may give a more accurate representation of tumour production. Furthermore, measurement of both IgGκ and IgGλ, calculation of the IgGκ/IgGλ ratio and comparison with values found in normal subjects can give a more sensitive indication of clonality[3]. Use of the IgGκ/IgGλ ratio will also compensate for any changes in plasma volume and correct for half life variations due to receptor saturation.

3 PRINCIPLE

Evaluating the concentration of a soluble antigen (e.g. IgG kappa) by turbidimetry involves the addition of the test sample to a solution containing the appropriate antibody (anti-IgG kappa) in a reaction vessel or cuvette. A beam of light is passed through the cuvette and, as the antigen-antibody reaction proceeds, the light passing through the cuvette is scattered increasingly as insoluble immune complexes are formed. Light scatter is monitored by measuring the decrease in intensity of the incident beam of light. The antibody in the cuvette is in excess so the amount of immune complex formed is proportional to the antigen concentration. A series of calibrators of known antigen concentration are assayed initially to produce a calibration curve of measured light scatter versus antigen concentration. Samples of unknown antigen concentration can then be assayed and the results read from the calibration curve.

4 REAGENTS

4.1 **Antiserum:** Consisting of polyclonal monospecific sheep antibody supplied in liquid form. It contains 0.099% sodium azide, 0.1% E-amino-n-caproic acid (EACA), 0.01% benzamidine and 1mM ethylenediaminetetraacetic acid (EDTA) as preservatives.
4.2 **Calibrators:** These consist of pooled normal human sera and are supplied in liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.
4.3 **Controls:** These consist of pooled human sera and are supplied in a stabilised liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.
4.4 **Reaction Buffer:** Contains 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 gloves, protective equipment and clothing at all times.** Only personnel fully trained in such methods should be permitted to perform these procedures.

This product contains sodium azide and must be handled with caution. 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 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 antiserum, reaction buffer, calibrators and controls may be stored for up to 3 months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. The antiserum and reaction buffer may be stored, uncapped, on the SPAPLUS analyser for up to 1 month, provided that the main power switch (located at the rear of the left hand panel) is left switched on.

7 SPECIMEN COLLECTION AND PREPARATION

Blood samples should be obtained by venepuncture, 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 21 days, but for prolonged storage they should be kept frozen at -20°C[4]. Repeated freeze/thaw cycles should be avoided. Microbially contaminated samples, samples containing particulate matter and lipaemic or haemolysed samples should not be used.
It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific sample stability criteria for its laboratory[5].

8 METHODOLOGY

Note: to enable improved interpretation of results, IgG kappa/IgG lambda ratios should be determined; samples must therefore also be assayed using Binding Site's **Hevylite** Human IgG Lambda SPAPLUS kit (NK622.S).

8.1 Materials provided

8.1.1 1 x 50 tests *Human IgG Kappa SPAPLUS Antiserum*
8.1.2 1 x *Human IgG Kappa SPAPLUS Calibrator Set (6 x 1.0mL)*
8.1.3 1 x 1.0mL *Human IgG Kappa SPAPLUS High Control*
8.1.4 1 x 1.0mL *Human IgG Kappa SPAPLUS Control*
8.1.5 1 x 50 tests *IgG Kappa SPAPLUS Reaction Buffer*

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 SPAPLUS Sample Diluent 2 (100: Dil 2) Pack Code: SN114.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 22.

Item Name 22 IgGK
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 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	[ ]	[ ]	[ ]	[ ]

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Item Name 22 IgGK

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 4
REAGENT1 VOL 200 µL
REAGENT2 VOL 60

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

MONITOR
0 LEVEL SPAN 1
SPAN 3

DATA PROCESS
READ

	START	END
MAIN	53	54
SUB	30	31

ABSORBANCE LIMIT
LOW -3
HIGH 3

FACTOR
Blank correction *
ENDPOINT LIMIT 2
LINEAR CHECK (%) 0

Reaction Check
○ ON ● OFF
CHECK POINT
LOW -3.0
HIGH 3.0

DILUTION
Diluent ○ 99: Dil 1 ●100: Dil 2
Pre Dilution Rate 20 ▼
Auto Rerun Dilution Rate High 80 ▼
Auto Rerun Dilution Rate Low ▼

PROZONE CHECK

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

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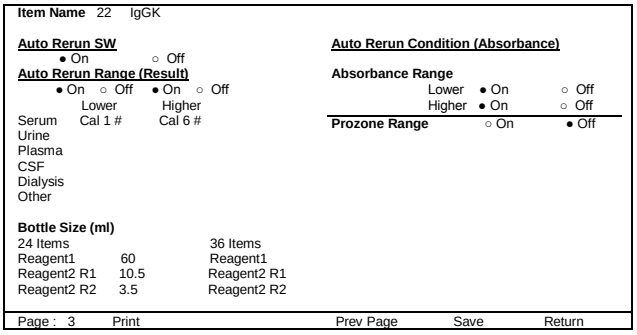

NB. The calibrator (Standard #) values are found in the Quality Control Certificate (SINS621.DS). Calibrator values on **Page 1** should be entered in ascending order, i.e. the lowest value first. The analyser will automatically calculate and enter the correct measuring ranges on item pages 3 and 4 providing 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.

8.4.2 The SPAPLUS can be set up to automatically calculate the IgG Kappa / IgG Lambda ratio. Refer to the SPAPLUS Reference Guide (FIN012) for details of installing calculation parameters.

8.5 Measuring range

All samples must be assayed first at the standard 1/20 sample dilution, giving an approximate measuring range of 1.9 – 40.0g/L. This enables a sensitivity of 0.094g/L on neat serum samples. The upper limit of the measuring range using a sample dilution of 1/80 is 160.0g/L. **Visibly turbid samples must be clarified by centrifugation before being assayed.**

Analyser dilution	Approximate range (g/L)
1/1	0.09 – 2.00
1/20	1.9 – 40.0
1/80	7.5 – 160.0

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 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 (SINS621.DS). 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 Decisions on patient evaluation and management must not be given on the basis of IgG kappa, IgG lambda or IgG kappa/IgG lambda ratio measurements alone. Clinical history and other laboratory findings must be taken into account.

10.3 Monoclonal immunoglobulins are highly variable. Any sample giving unexpected results should be retested at a higher dilution (lower concentration) to preclude antigen excess.

10.4 The effect of therapeutic drugs on the measurement of IgG kappa by this assay has not been evaluated.

11 EXPECTED VALUES

The ranges below were obtained by measuring the IgG kappa and IgG lambda concentrations of 129 normal sera and are intended for guidance purposes only. Wherever possible it is strongly recommended that local ranges are generated.

Normal adult serum	Mean	Median	95 Percentile Range
IgG kappa (g/L)	7.10	6.75	3.84-12.07
IgG lambda (g/L)	3.95	3.90	1.91-6.74
IgG kappa/ IgG lambda ratio	1.84	1.74	1.12-3.21

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A precision study was performed following CLSI *Evaluation of Precision Performance of Clinical Chemistry Approved Guideline* (CLSI Document EP5-A). The study was carried out over 21 working days, with two runs per day. One user assessed three different samples using three different reagent lots on one analyser. The following values were obtained for the samples tested:

IgG kappa Precision Summary									
	Mean (g/L)	Within run		Between run		Between-day		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
Serum 1	25.18	0.32	1.2	0.52	2.0	1.53	5.7	1.65	6.1
Serum 2	9.69	0.11	1.2	0.25	2.6	0.43	4.4	0.51	5.2
Serum 3	3.18	0.07	2.3	0.13	4.1	0.16	5.3	0.22	7.1

12.2 Comparison

Serum samples from 129 normal individuals were tested using the **Hevylite** IgG kappa and IgG lambda kits and total IgG was measured using the Human IgG SPAPLUS kit. The sum of **Hevylite** (IgG kappa plus IgG lambda) was compared with total IgG (range 4.5 - 18.4g/L):

y = 0.97x + 0.44g/L (Passing-Bablok comparison)

Serum samples from 117 multiple myeloma patients (heavy chain and light chain types classified by immunofixation) were tested using the **Hevylite** IgG kappa and IgG lambda kits, monoclonal IgG was measured using serum protein electrophoresis densitometry. **Hevylite** (IgG kappa or IgG lambda) was compared with monoclonal IgG (range 3.57 - 112.5g/L):

y = 1.06x - 1.78g/L (Passing-Bablok comparison)

12.3 Limit of Blank and Limit of Detection

The limit of blank is calculated from the mean blank concentration (n=60) plus 2 standard deviations, which is equivalent to 0.04g/L at the minimal sample dilution (1/1). The limit of detection represents the lowest measurable concentration of analyte that can be distinguished from zero; it has been calculated as 0.05g/L (n=60) using a low concentration serum at the minimal sample dilution. The limit of quantitation for this assay is 0.09g/L, calculated from the lowest calibrator concentration divided by the minimal sample dilution.

12.4 Linearity

The linearity of this assay has been confirmed using a serially diluted serum sample, which gave a regression plot of y = 0.987x - 0.42g/L, r^2 = 0.999 (y = measured IgG kappa concentration, x = theoretical concentration), over a measuring range of 3.48 - 34.8g/L.

12.5 Interference

Minimal assay interference by 200mg/L bilirubin (-2.0%), 4.56g/L haemoglobin (-1.3%) and 1540 FTU chyle (1.6%) has been demonstrated using a 0.71g/L IgGκ serum at the minimum sample dilution (1/1). No cross-reactivity was seen with monoclonal samples containing IgG lambda, IgA kappa, IgA lambda, IgM kappa, IgM lambda, free kappa and free lambda. Interference by therapeutic drugs has not been tested.

13 BIBLIOGRAPHY

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4. The WHO document "Use of Anticoagulants in Diagnostic Laboratory Investigations" (WHO/DIL/LAB/99.1 Rev. 1)
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