# Hevylite® Human IgM Kappa Kit for use on the SPAPLUS®

## For *in vitro* diagnostic use

## Product code: NK625.S

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## 1 INTENDED USE

This kit is intended for the *in vitro* quantification of IgM 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 IgM concentration ranges from 0.5 - 2.0 g/L[1].
Elevated serum concentrations of monoclonal protein are indicative of an underlying abnormality such as monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, Waldenström's Macroglobulinaemia and other lymphoproliferative disorders. Serum protein electrophoresis (SPE) densitometry is recommended to quantify monoclonal proteins[2]. Turbidimetry can also be used in these instances to measure total IgM but this will include non-tumour immunoglobulin, and measurement of either IgMκ or IgMλ may give a more accurate representation of tumour production. Use of the IgMκ/IgMλ ratio will also compensate for any changes in plasma volume and haematocrit.

## 3 PRINCIPLE

Evaluating the concentration of a soluble antigen (e.g. IgM kappa) by turbidimetry involves the addition of the test sample to a solution containing the appropriate antibody (anti-IgM 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** **Latex Reagent:** Consisting of polyclonal monospecific sheep antibody coated onto polystyrene latex. Preservative: 0.05% ProClin™, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine 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.

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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 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 ProClin 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 reagent, reaction buffer, calibrators and controls may be stored for up to 1 month after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator.
The reagent 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[3]. 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[4].

## 8 METHODOLOGY

**Note:** to enable improved interpretation of results, IgM kappa/IgM lambda ratios should be determined; samples must therefore also be assayed using Binding Site's **Hevylite** Human IgM Lambda SPAPLUS kit (NK626.S).

### 8.1 Materials provided

8.1.1 1 x 50 tests *Human IgM Kappa SPAPLUS Reagent*
8.1.2 1 x *Human IgM Kappa SPAPLUS Calibrator* (6 x 1.0mL)
8.1.3 1 x 1.0mL *Human IgM Kappa SPAPLUS High Control*
8.1.4 1 x 1.0mL *Human IgM Kappa SPAPLUS Control*
8.1.5 1 x 50 tests *IgM 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) Product 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 26.**

**Item Name** 26 IgMK

**DATA INFORMATION**
**Units** g/L
**Decimals** 3

**ANALYSIS**
Type End ▼

Main W.Length 1 505 ▼
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** 26 IgMK

**ASPIRATION**
**KIND** ○ Single ● Double
**VOLUME**
**SAMPLE** 3
**REAGENT1 VOL** 120 µL
**REAGENT2 VOL** 100

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

**MONITOR**
0 LEVEL SPAN 1
SPAN 3

**DATA PROCESS**
**READ** **ABSORBANCE LIMIT**

| | START | END | | |
|---|---|---|---|---|
| MAIN | 53 | 54 | LOW | -3 |
| SUB | 35 | 36 | HIGH | 3 |

**FACTOR** **Reaction Check**
Blank correction 1 ○ ON ● OFF
ENDPOINT LIMIT 2 CHECK POINT
LINEAR CHECK (%) 0 LOW -3.0
HIGH 3.0

**DILUTION**
Diluent ○ 99: Dil 1 ●100: Dil 2
Pre Dilution Rate 10 ▼
Auto Rerun Dilution Rate High 90 ▼
Auto Rerun Dilution Rate Low ▼

**PROZONE CHECK**

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

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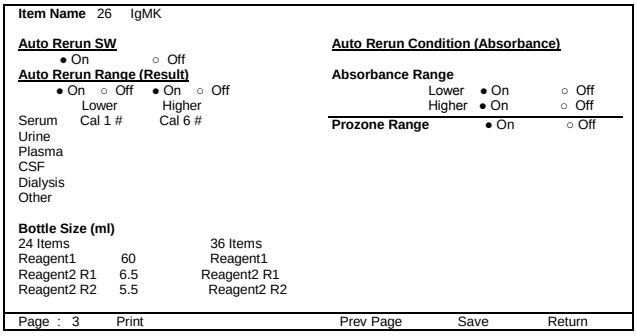

**Item Name** 26 IgMK

**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 | 6.5 | Reagent2 R1 |
| Reagent2 R2 | 5.5 | Reagent2 R2 |

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**N.B.** The calibrator (Standard #), prozone check (#) and Min dOD (#) values are found in the quality control certificate (SINS625.DS). Calibrator values on **Page 1** should be entered in ascending order, i.e. the lowest value first. The prozone check and Min dOD values (#) should be entered on **Page 2**. 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.

8.4.2 The SPAPLUS can be set up to automatically calculate the IgM Kappa / IgM 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 1/10 sample dilution, giving an approximate measuring range of 0.2 – 5.0g/L. This enables a sensitivity of 0.02g/L on neat serum samples. The upper limit of the measuring range using a sample dilution of 1/90 is 45.0g/L. For samples measuring over this limit the following dilution series should be used. **Visibly turbid samples must be clarified by centrifugation before being assayed.**

| Overall dilution | Analyser dilution | Manual pre-dilution | Approximate range (g/L) |
|---|---|---|---|
| 1/1 | 1/1 | - | 0.02 – 0.50 |
| 1/10 | 1/10 | - | 0.20 – 5.00 |
| 1/90 | 1/90 | - | 1.80 – 45.00 |
| 1/250* | 1/10* | 1/25* | 5.00 – 125.00 |

* Make a manual pre-dilution of 1/25 by taking 40µL of sample and adding 960µL of Sample Diluent 2. Present the 1/25 diluted sample for analysis at 1/10. Multiply the result by 25.

### 8.6 Interpretation of results

The results of this assay should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings including previous **Hevylite** IgM kappa results if available.

Due to the nature of monoclonal proteins, some samples may exhibit non linearity when assayed at different dilutions. In order to appropriately quantify such samples it is advised that the dilution protocol described in section 8.5 is followed and the first plausible result is reported.

All immunoassays have the potential for antigen excess. In order to identify samples that are in antigen excess the SPAPLUS has the facility to monitor reaction kinetics. Samples that demonstrate unusual reaction kinetics will generate a P flag. Samples that have generated a P flag must be repeated at a higher dilution as described in section 8.5 (note: the SPAPLUS will carry out auto-dilutions up to 1/90). If upon repeat the sample gives a result that is considered implausible, the samples should be repeated at the initial dilution, reviewed and reported.

Refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser for further details of flag interpretation.

**Important note:** No automated check will identify all cases of antigen excess and a very small percentage of samples in antigen excess may not prompt the "P" flag. It is recommended that the following statement accompany all **Hevylite** IgM kappa results.

*"Undetected antigen excess is a rare event but cannot be excluded. If the **Hevylite** IgM kappa 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 other laboratory tests and clinical evidence; any anomalies should be discussed with the testing laboratory."*

## 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 SPA PLUS 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 (SINS625.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 IgM kappa, IgM lambda or IgM kappa/IgM lambda ratio measurements alone. Clinical history and other laboratory findings must be taken into account.

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

## 11 EXPECTED VALUES

The ranges below were obtained by measuring the IgM kappa and IgM lambda concentrations of 147 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 |
|---|---|---|---|
| IgM kappa (g/L) | 0.71 | 0.63 | 0.19 – 1.63 |
| IgM lambda (g/L) | 0.39 | 0.35 | 0.12 – 1.01 |
| IgM kappa/ IgM lambda ratio | 1.85 | 1.81 | 1.18 – 2.74 |

## 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:

| IgM kappa Precision Summary | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean (g/L) | Within run | | Between run | | Between-day | | Total | |
| | | SD | CV% | SD | CV% | SD | CV% | SD | CV% |
| Serum 1 | 4.13 | 0.08 | 1.8 | 0.08 | 1.8 | 0.21 | 4.6 | 0.24 | 5.3 |
| Serum 2 | 1.80 | 0.03 | 1.5 | 0.02 | 1.3 | 0.07 | 3.5 | 0.08 | 4.1 |
| Serum 3 | 0.34 | 0.01 | 2.4 | 0.01 | 3.3 | 0.02 | 4.9 | 0.02 | 6.4 |

### 12.2 Comparison

Serum samples from 147 normal individuals and 47 IgM monoclonal protein patients (heavy chain and light chain types classified by immunofixation) were tested using the **Hevylite** IgM kappa and IgM lambda kits. Total IgM was measured using the Human IgM SPAPLUS kit. The sum of **Hevylite** (IgM kappa plus IgM lambda) was compared with total IgM (range 0.24 – 62.69g/L):

For 147 normal sera (range 0.24 – 3.32g/L)

$$y = 0.96x + 0.08 g/L \text{ (Passing-Bablok comparison)}$$

For 147 normal sera and 47 patient samples (range 0.24 - 62.69g/L)

$$y = 1.01x + 0.04 g/L \text{ (Passing Bablok comparison)}$$

All 25 IgM kappa patient samples had IgM kappa / IgM lambda ratios above the quoted 95 percentile range, and all 22 of the IgM lambda patient samples had ratios below the 95 percentile range.

### 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 0.001g/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 0.009g/L (n=60) using a low concentration serum at the minimal sample dilution. The limit of quantitation for this assay is 0.020g/L, calculated from the lowest calibrator concentration.

### 12.4 Linearity

The linearity of this assay has been confirmed using a serially diluted serum sample, which gave a regression plot of $y = 0.992x - 0.144 g/L$, $r^2 = 0.995$ (y = measured IgM kappa concentration, x = theoretical concentration), over a measuring range of 0.15 – 5.37g/L.

### 12.5 Interference

Minimal assay interference by 200mg/L bilirubin (2.6%), 5.0g/L haemoglobin (-1.8%) and 1500 FTU chyle (2.2%) has been demonstrated using a 0.179g/L IgM kappa serum at the minimum sample dilution (1/1). No cross-reactivity was seen with monoclonal samples containing IgG kappa, IgG lambda, IgA kappa, IgA lambda, IgM lambda, free kappa and free lambda. Interference by therapeutic drugs has not been tested.

## 13 BIBLIOGRAPHY


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2. Kyle, R.A. & Rajkumar, S.V. (2003a) Monoclonal gammopathies of undetermined significance: a review. Immunological Reviews, 194, 112-139.
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4. CLSI GP44-A4, Vol. 30 No. 10, 5.5.1.1.1, May 2010, "Procedures for the handling and processing of blood specimens for common laboratory tests; Approved Guideline"