Human IgM kit for use on SPAPLUS®

For *in vitro* diagnostic use only

Product Code: NK012.S

Product manufactured by:
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FDA (USA) Information:
Analyte Name: Immunoglobulin IgM
Complexity Cat.: Moderate

CE

1 INTENDED USE

This kit is intended for the quantitative *in vitro* determination of human IgM in human serum, lithium heparin or EDTA plasma, using the Binding Site SPAPLUS turbidimetric analyser. Measurement of IgM aids in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents. The test results are to be used in conjunction with other clinical and laboratory findings.

2 SUMMARY AND EXPLANATION

IgM is the first class of immunoglobulin synthesised in response to particulate antigens. Each unit consists of two mu heavy chains and two light chains, five units together with a J-chain comprising an IgM molecule. IgM is therefore multivalent and deals most efficiently with polyvalent antigens such as bacteria and viruses. IgM also activates complement. On active immunisation IgM rapidly appears in serum, but levels normally drop after a week, usually in parallel with the increase in IgG. Normal serum levels are dependent on age. Raised serum levels are associated with hepatitis, myeloma, Waldenström's macroglobulinaemia and other infections. Reduced levels can occur in antibody deficiency syndrome (ref. 1).

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 IgM 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 IgM 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 cleared 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 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 IgM Antiserum and Reaction Buffer 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 (ref. 2). 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 14 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 IgM Antiserum*
8.1.2 1 x *Human IgM SPAPLUS Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1mL *Human IgM SPAPLUS High Control*
8.1.4 2 x 1mL *Human IgM SPAPLUS Low Control*
8.1.5 1 x 100 Tests *IgM 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 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 15.

Item Name 15 IgM
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 Auto Fill
Type Spline 1▼
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 15 IgM

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 30
REAGENT1 VOL 160 µL
REAGENT2 VOL 40

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 *

Reaction Check
○ ON ● OFF
ENDPOINT LIMIT 2.0
LINEAR CHECK (%) 0
CHECK POINT
LOW -3
HIGH 3

DILUTION
Diluent ● 99: Dil 1 ○ 100: Dil 2
Pre Dilution Rate 20 ▼
Auto Rerun Dilution Rate High 40 ▼
Auto Rerun Dilution Rate Low 10 ▼

PROZONE CHECK

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

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*Automatically calculated

Item Name 15 IgM

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
Reagent2 R1	17	Reagent2 R1
Reagent2 R2	5	Reagent2 R2

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 (SIN144.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

All samples must be assayed first at the standard 1/20 sample dilution, giving an approximate measuring range of 0.2-7.5g/L. Samples higher than this will automatically re-dilute using a 1/40 dilution, which allows up to 15g/L to be measured. Samples measuring above this range should be manually diluted as detailed in the table below.

Overall dilution	Analyser dilution	Manual pre-dilution	Approximate range (g/L)
1/10	1/10	-	0.1 – 3.8
1/20	**1/20**	**-**	**0.2 – 7.5**
1/40	1/40	-	0.4 - 15
1/200	1/20	1/10	2 - 75
1/400	1/40	1/10	4 - 150

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 (SIN144.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.

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 IgM measurements alone. Clinical history and other laboratory findings must be taken into account.

10.4 Potential occurrences of antigen excess cannot be completely excluded; in rare cases samples with monoclonal IgM present may give falsely low results due to antigen excess. Where this is possible or suspected it is recommended that the sample is re-assayed at a higher dilution to confirm the result.

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. During childhood and adolescence reference ranges for IgM are dependent on age and can vary over a wide range.

Adult serum ranges

These ranges were obtained using this kit, by measuring the IgM 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)
IgM	120	1.02	0.85	0.35-2.42

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.

IgM Precision Summary									
	Mean (g/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	0.36	0.008	2.3	0.01	3.0	0.02	4.9	0.02	6.2
Serum 2	2.79	0.018	0.6	0.02	0.8	0.10	3.8	0.11	3.9
Serum 3	6.44	0.038	0.6	0.08	1.3	0.10	1.6	0.14	2.1

12.2 Comparison

A correlation study was performed on 71 samples (42 normal serum, 26 clinical serum and 3 clinical plasma samples) using this kit on a SPAPLUS and an alternative commercially available IgM assay. The study demonstrated excellent agreement with the following Passing Bablok plot:

y = 1.01x - 0.02 (g/L) (y = SPAPLUS IgM; x = alternative assay)

correlation coefficient r = 0.9944 (calculated by linear regression)

12.3 Limit of Blank and Limit of Detection

The limit of blank is calculated as the mean blank (n = 60) plus 2 standard deviations. This is equivalent to a concentration of 0.001g/L.
The limit of detection represents the lowest measurable analyte level that can be distinguished from zero. This has been estimated at 0.004g/L (n = 60).
The limit of quantitation for this assay is defined as the lowest point of the calibration curve (i.e. 0.2g/L based upon a 1/20 sample dilution).

12.4 Linearity

The linearity of this assay has been confirmed using serially diluted serum samples, giving a regression equation of *y=0.9965x – 0.0004 (g/L), r =0.999,* (y = measured IgM, x = theoretical concentration) over the range of 0.24 - 6.29g/L.

12.5 Interference

No significant assay interference by 2820 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, 4.82g/L haemoglobin has been demonstrated at the minimum sample dilution (1/10).

	Bilirubin	Hb	Chyle (FTU)
Mean IgM (g/L)	0.237	0.228	2.73
% interference	-0.4	-1.7	-2.7

No significant interference was observed with a serum sample containing 600 IU/mL Rheumatoid Factor (RF).

12.6 Antigen excess

No antigen excess was observed to a level of eight times the top point of the assay; approximately 56.4g/L. In rare cases samples may exhibit antigen excess below this level - see section 10.4.

13 BIBLIOGRAPHY

1. Zilva, JF & Pannall, PR (1984) Clinical Chemistry in Diagnosis and Treatment. Publ. Lloyd–Luke (Medical Books) Ltd, London pp 348-352.
2. Use of Anticoagulants in Diagnostic Laboratory Investigations WHO/DIL/LAB/99.1 Rev.2 2002.