Human IgD Kit for use on SPAPLUS®

For *in vitro* diagnostic use only

Product Code: LK013.S

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FDA (USA) Information:
Analyte Name: IgD
Complexity Cat.: Moderate

CE

1 INTENDED USE

This kit is intended for measuring human immunoglobulin D (IgD) in serum as an aid in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents. The test result is to be used in conjunction with other clinical and laboratory findings.

2 SUMMARY AND EXPLANATION

IgD, molecular weight 185kD (ref. 1), is one of the five classes of human immunoglobulin. It is present on the surface of the majority of circulating B lymphocytes, indicating that the virgin B cell is ready for priming by antigen. The IgD is lost on antigenic stimulation, therefore memory cells lack this immunoglobulin. IgD is a functionally significant protein but its precise role is unknown, although there are suggestions that it is primarily a cell-surface antigen receptor (triggering lymphocyte differentiation) and a ligand for IgD receptors on immunoregulatory helper T cells. It is very susceptible to proteolysis (ref. 4). IgD accounts for less than 1% (ref. 1) of the total plasma immunoglobulin. Serum concentrations are influenced by age and inheritance. Very high serum IgD concentrations are found in IgD myeloma patients. IgD levels are also found to be raised in Hyperimmunoglobulineamia D syndrome (HIDS), an autosomal recessive disorder characterised by recurrent febrile attacks with abdominal, articular, and skin manifestations (refs 2, 3, 4).

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.

Some antibody antigen complexes do not form sufficiently large immune complexes to be detected by turbidimetry. If antibody is coated onto latex particles of a suitable size, the light scattering ability of the immune complexes formed is improved significantly allowing turbidimetric detection.

4 REAGENTS

4.1 **Human IgD latex reagent** This is a monospecific antiserum coated onto polystyrene latex and supplied in stabilised liquid form. Contains 0.033% sodium azide, 0.1% E-amino-n-caproic acid (EACA), 0.01% benzamidine and 0.05% ProClin™* 300 as preservatives.

4.2 **Calibrators and controls** These consist of pooled human serum and are supplied in stabilised liquid form. The assay is calibrated to Human Serum Immunoglobulin D British Research Standard NIBSC 67/037. Contain: 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.

4.3 **Reaction Buffer** Contains 0.099% sodium azide as a preservative.

** ProClin 300 is a trademark of Rohm and Haas corp., Philadelphia, PA.*

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, provided that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. The Human IgD reagent and Reaction Buffer may be stored, uncapped, on the SPAPLUS analyser for up to 28 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 samples. Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 2-8°C for up to 48 hours prior to assay, or for prolonged storage kept at -20°C or below. If prolonged storage (-20°C) is required it is recommended that a preservative and a protease inhibitor should be added to the sample to prevent deterioration (for example benzamadine and sodium azide (ref. 4)). Repeated freezing and thawing should be avoided. Microbially contaminated, haemolysed and lipaemic serum and samples containing particulate matter should not be used.

8 METHODOLOGY

8.1 Materials Provided

8.1.1 1 x 100 Tests *Human IgD Latex Reagent for SPAPLUS*
8.1.2 1 x *Human IgD SPAPLUS Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1mL *Human IgD High Control for SPAPLUS*
8.1.4 2 x 1mL *Human IgD Low Control for SPAPLUS*
8.1.5 1 x 100 Tests *IgD Reaction Buffer for SPAPLUS*

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 16.

Item Name 16 IgD
DATA INFORMATION
Units mg/L
Decimals 3

ANALYSIS
Type End ▼

Main W.Length 1 600 ▼
Sub W.Length ▼

Method

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION AutoFill
Type Logit 2 ▼
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 16 IgD

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 7
REAGENT1 VOL 145 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 *
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 40 ▼
Auto Rerun Dilution Rate Low ▼

PROZONE CHECK

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

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*Automatically calculated

Item Name 16 IgD

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	15.5	Reagent2 R1
Reagent2 R2	9	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 #), prozone check (#) and Min dOD (#) values are found in the quality control certificate (SIN169.QC). 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.'

8.5 Measuring range

The approximate measuring range of the IgD assay when using the standard 1/10 sample dilution is 7 – 210mg/L. Where results are greater than the measuring range, samples should be manually diluted offline at 1/20 and rerun at 1/10 to give an overall dilution of 1/200. If the sample is still over range, the user may manually select an on-line 1/40 dilution to give an overall 1/800 dilution.

Off-line dilution	On-line dilution	Approximate measuring range
	1/10	7- 210mg/L
1/20	1/10	140 - 4200mg/L
	1/40	560 – 16800mg/L

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 IgD 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 isadvised 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. 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 (i.e. a small proportion of samples containing monoclonal IgD) in antigen excess may not prompt the "P" flag. It is recommended that the following statement accompany all IgD results.

Undetected antigen excess is a rare event but cannot be excluded, If the IgD 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; 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 SPAPLUS Operation Manual.

9.2 Quality control testing should be performed in accordance with regularatory 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 (SIN169.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 IgD 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. During childhood and adolescence, reference ranges for IgD are dependent on age and can vary over a wide range.

Adult serum ranges

These ranges were obtained using this kit, by measuring the IgD concentration of sera taken from healthy adult US 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)
IgD	120	49.4	42.8	7.7-132.1

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A study was performed following CLSI *Evaluation of Precision Performance of Clinical Quantitative Measurement Methods; Approved Guideline* (CLSI Document EP5-A2)**.** 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.

IgD Precision Summary									
	Mean	Within run		Between run		Between day		Total	
	(mg/L)	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	169.42	2.50	1.5	1.86	1.1	9.90	5.8	10.38	6.1
Serum 2	122.60	1.44	1.2	1.14	0.9	5.62	4.6	5.92	4.8
Serum 3	11.66	0.26	2.3	0.25	2.2	1.08	9.2	1.14	9.8

12.2 Comparison

A correlation study was performed on 97 samples (50 normal serum, 15 IgD myeloma serum and 32 other clinical samples) using this kit on a SPAPLUS and an alternative commercially available IgD assay. The study demonstrated excellent agreement with the following Passing Bablok plot:

y = 0.95x - 0.59 (mg/L) (y = SPAPLUS IgD; x = alternative assay)

correlation coefficient r = 0.995 (calculated by linear regression)

12.3 Limit of Blank and Limit of Detection

Based on *CLSI document EP17-A - Protocols for Determination of Limits of Detection and Limits Quantitation; Approved Guideline* the limit of detection represents the lowest measurable analyte level that can be distinguished from zero. This has been estimated at 0.4mg/L (n = 60).
The limit of quantitation for this assay is defined as the lowest point of the calibration curve i.e. 7mg/L based upon a 1/10 sample dilution.

12.4 Linearity

A linearity study was performed following CLSI *Evaluation of the Linearity of Quantitative Measurement Procedures* (CLSI Document EP6-A). One user assessed the linearity of a pool of high samples diluted in a pool of low samples using one lot of reagent on one analyser. This gave a regression plot of $y = 0.9988x – 4.724$, $R^2 = 0.9946$ (y = measured IgD concentration, x = theoretical concentration).

12.5 Interference

No significant assay interference by 1560 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, 4.8g/L haemoglobin has been demonstrated at the minimum sample dilution (1/10).

	Bilirubin	Hb	Chyle (FTU)
Mean IgD (mg/L)	6.55	6.83	6.66
% interference	-0.5%	0.0%	-1.0%

No cross reaction is seen with IgA, IgG or IgM at a standard 1/10 sample dilution.

12.6 Antigen excess

Antigen excess was not seen in samples up to 1.68g/L (1680mg/L) when tested with a polyclonal serum sample, for further information on antigen excess however please refer to section 8.6.

13 BIBLIOGRAPHY

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