Optilite®
OPTIMISED PROTEIN SYSTEM

Optilite® IgG2 Kit

For *in-vitro* diagnostic use only

Product Code: NK007.OPT

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1 INTENDED USE

The Optilite IgG2 Kit is intended for the quantitative *in vitro* measurement of IgG2 in serum, lithium heparin and EDTA plasma using the Binding Site Optilite analyser. Measurement of this immunoglobulin is an aid in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents. This test should be used in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

In normal adults, IgG constitutes approximately 75% of the total serum immunoglobulin. Within the IgG class, the usual order of concentration of the 4 subclasses is IgG1>IgG2>IgG3>IgG4, but the actual concentration of each may vary markedly between individuals. The four IgG subclasses show considerable differences in their properties, including ability to fix complement, to bind to macrophages and to pass through the placenta. Abnormal levels of one or more subclass may be associated with certain conditions, including anaphylaxis, autoimmune- and gut diseases as well as hypo- and hyper-gammaglobulinaemia (Ref. 1). In particular, reduced production of IgG2 in children may be associated with recurrent infections (Ref 2). The subject has been reviewed (Refs 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.

4 REAGENTS

4.1 **Antiserum:** Supplied in stabilised liquid form. Preservatives: 0.099% sodium azide, 0.1% E-amino-n-caproic acid (EACA), 1mM ethylenediamine-tetraacetic acid (EDTA) and 0.01% benzamidine.

4.2 **Calibrator and Controls:** Pooled human serum, supplied in stabilised liquid form. Containing 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. The concentration given on the quality control certificate has been obtained by comparison with the DA470k international reference material (Refs 5, 6).

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.

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, Calibrator 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 Reagent may be stored uncapped on the Optilite analyser for up to 30 days, provided that the power is left switched on.

7 SPECIMEN COLLECTION AND PREPARATION

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. Sera may be stored at 2-8°C for up to eight days, otherwise aliquot and freeze undiluted at -20°C or below for prolonged storage. Repeated freezing and thawing should be avoided. Centrifuge samples containing precipitates before performing the assay. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory (Ref 7).

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 100 Tests *Optilite IgG2 Reagent*
8.1.2 1 x 2.0mL *Optilite IgG2 Calibrator*
8.1.3 1 x 1.5mL *Optilite IgG2 High Control*
8.1.4 1 x 1.5mL *Optilite IgG2 Low 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 Optilite analyser.
8.2.3 Current analyser operating instructions: Optilite Operation Manual, Insert Code INS700.OPT
8.2.4 Optilite Diluent 1, Product Code IK709
8.2.5 Optilite Diluent 2, Product Code IK710

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 Optilite analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the instruction in the Optilite Operation Manual.

8.4.1 Assay parameters for this assay are provided as barcodes on the accompanying QC certificate (QCcert007.OPT). Scan Barcode 1 and Barcode 2 to load the parameters.

8.5 Measuring range

The approximate measuring range of the assay is shown in the table below.

Optilite Analyser Dilution	Approximate range (mg/L)
1+0	20 – 700
1+9	**200 – 7000**
1+ 39	800 – 28000

9 QUALITY CONTROL

At least two levels of appropriate controls 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 Optilite Operation Manual.

Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

The concentrations of the controls are stated on the accompanying QC certificate (QCcert007.OPT). Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.

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.

A value for summated IgG is obtained by adding the values of the four IgG subclasses together and should be within ±20% of the total IgG (see 10.4).

10 LIMITATIONS

10.1 Turbidimetric assays are not suitable for measurement of samples containing rheumatoid factor or paraproteins, 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 Diagnosis cannot be made and treatment must not be given on the basis of IgG2 measurements alone. Clinical history and other laboratory findings must be taken into account.
10.3 The results obtained from measuring IgG subclasses should not be used in assessing atopy in allergic patients.
10.4 If the IgG summation (see section 9) is outside the acceptable range samples should be repeated at a higher dilution.
10.5 Potential occurrences of antigen excess cannot be completely excluded; in rare cases samples with monoclonal IgG2 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.

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10.6 The "Blank Resp high" flag indicates that the sample is turbid. Any sample that produces this flag should be visually examined and if necessary centrifuged and re-assayed. Lipaemic samples are known to interfere with this assay and should not be analysed.

11 EXPECTED VALUES

The ranges provided have been obtained from a limited number of samples and are intended for guidance purposes only. Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population and, if necessary, determine its own reference interval.

Adult serum range

	Number (n)	Mean (mg/L)	Median (mg/L)	95 Percentile Range (mg/L)
IgG2	30	4528	4541	2418 - 7003

Paediatric serum ranges

These ranges were obtained by measuring the IgG2 concentration of paediatric serum samples from a Birmingham Hospital using Binding Site Radial Immunodiffusion products. All concentrations are in mg/L.

Age	Number (n)	Mean (mg/L)	95 Percentile Range (mg/L)
0-2 years	39	838	225 – 3000
2-4 years	36	1146	360 – 2250
4-6 years	49	1496	605 – 3450
6-8 years	43	1754	440 – 3750
8-10 years	32	2126	720 – 4300
10-12 years	46	1980	760 – 3550
12-14 years	54	2486	1000 – 4550
14-18 years	48	2614	640 – 4950

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

The precision study was based on CLSI EP5-A2 *Evaluation of Precision Performance of Clinical Quantitative Measurement Methods.* The study was performed over 21 working days, with 2 runs per day. One user assessed 7 different samples, using 1 reagent lot on 4 analysers.

Precision Summary									
	Mean (mg/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Level 1*	80.4	1.2	1.5	1.9	2.4	3.1	3.9	3.9	4.8
Level 2*	417.4	3.0	0.7	4.4	1.0	10.7	2.6	11.9	2.9
Level 3	389.5	12.9	3.3	6.1	1.6	17.1	4.4	22.3	5.7
Level 4	1827.6	17.4	1.0	24.4	1.3	55.6	3.0	63.1	3.5
Level 5	2982.2	29.1	1.0	42.7	1.4	58.7	2.0	78.2	2.6
Level 6	5855.2	67.7	1.2	81.2	1.4	134.6	2.3	171.2	2.9
Level 7**	5942.8	104.5	1.8	78.7	1.3	185.5	3.1	227.0	3.8

***** performed at the 1+0 sample dilution
****** performed at the 1+39 sample dilution

12.2 Comparison

A comparison study was performed by analysing 333 samples (including 307 clinical sera and 26 normal sera) using the Optilite IgG2 kit and an alternative commercially available assay. Passing Bablok regression analysis generated the following results:

y = 0.99x – 10.0 (mg/L) (y = Optilite; x = predicate analyser)

correlation coefficient r = 0.995 (calculated by linear regression)

A comparison study was performed by analysing 52 paired serum and lithium heparin plasma samples using the Optilite IgG2 assay. Passing Bablok regression analysis generated the following results:

y = 1.00x - 30.0 (mg/L) (y = Li Hep plasma; x = serum)

correlation coefficient r = 0.993 (calculated by linear regression)

A comparison study was performed by analysing 52 paired serum and EDTA plasma samples using the Optilite IgG2 assay. Passing Bablok regression analysis generated the following results:

y = 0.97x + 30.0 (mg/L) (y = EDTA plasma; x = serum)

correlation coefficient r = 0.984 (calculated by linear regression)

12.3 Limit of Quantitation

The limit of quantitation (LoQ) for this assay is defined as the bottom of the measuring range, 20mg/L. The LoQ validation study was based on CLSI EP17-A *Protocols for Determination of Limits of Detection and Limits Quantitation.*

12.4 Linearity

The linearity study was based on CLSI EP6-A *Evaluation of the Linearity of Quantitative Measurement Procedures*. Linearity over the analyte range 188mg/L to 7773mg/L using the 1 + 9 sample dilution has been demonstrated.

Regression equation: y = 0.9612x + 29.8 (y = measured concentration, x = theoretical concentration); $r^2 = 0.9878$.

12.5 Interference

A study was performed following CLSI EP7-A2: Interference Testing in Clinical Chemistry, Approved Guideline (CLSI Document EP7-A2). A normal serum sample, serum samples close to the medical decision points and abnormal serum samples were tested. No significant assay interference effects were observed when tested with bilirubin (200mg/L) or haemoglobin (5g/L). Interference was observed at levels above 250mg/dL Intralipid and 500mg/dL Triglyceride, and lipaemic samples are known to interfere with this assay. Therefore lipaemic samples should not be analysed using this assay (see Section 10.6 for details).

No significant interference from commonly used therapeutic drugs is known. Further information is provided in literature (Ref 8).

12.6 Antigen excess

No antigen excess was observed up to a level of 5.1 times the top of the calibration curve at the standard 1 + 9 sample dilution. This is equivalent to 35970mg/L. In rare cases samples may exhibit antigen excess below this level - see section 10.5.

13 BIBLIOGRAPHY

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