Optilite® C4 Kit

For *in-vitro* diagnostic use only

Product Code: NK025.OPT

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CE

1 INTENDED USE

The Optilite C4 Kit is intended for the quantitative *in vitro* measurement of C4 in serum, lithium heparin or EDTA plasma using the Binding Site Optilite analyser. This test should be used in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

C4 is a component of the complement system, and has a fundamental role in the inflammatory response and immune system functionality. C4 is produced in the liver, and accordingly reduced complement activity is associated with severe liver failure (Ref 1). Deficiencies in C4 manifest in recurrent staphylococcal and streptococcal infections, and immune complex disorders such as glomerulonephritis, vasculitis, and endocarditis (Ref 2). Inherited or acquired deficiencies in C1q inhibitor cause decreased serum levels of C4, which are associated with angioedema (Ref 3).

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) and 0.01% benzamidine.

4.2 **Calibrator and Controls:** Pooled human serum, supplied in stabilised liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. The concentration of C4 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 urgently 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

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 three days, otherwise aliquot and freeze at -20°C or below and store for up to 3 months. Repeated freeze/thaw cycles should be avoided. Sample dilutions should be freshly prepared on the day of assay. **Note:** Upon storage, C4 degrades. Depending on the storage conditions, the C4 amounts in fresh samples are higher than in aged samples. Microbially contaminated, haemolysed and lipaemic serum and samples containing particulate matter should not be used. 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 4).

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 100 Tests *Optilite C4 Reagent*
8.1.2 1 x 2.1mL *Optilite C4 Calibrator*
8.1.3 1 x 1.5mL *Optilite C4 High Control*
8.1.4 1 x 1.5mL *Optilite C4 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 (QCcert025.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 (g/L)
1+0	0.0064 – 0.09
1+9	**0.064 – 0.9**
1+19	0.128 –1.8

9 QUALITY CONTROL

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 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 provided are stated on the accompanying QC certificate (QCcert025.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.

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 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.
10.3 Diagnosis cannot be made and treatment must not be given on the basis of C4 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. 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 (g/L)	Median (g/L)	95 Percentile Range (g/L)
C4	120	0.241	0.234	0.129 - 0.392

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 5 different samples, using 1 reagent lot on 3 analysers.

Precision Summary									
	Mean	Within run		Between run		Between day		Total	
	(g/L)	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Level 1*	0.019	0.0004	2.4	0.0005	2.8	0.0006	3.2	0.0009	4.9
Level 2	0.079	0.003	3.9	0.0033	4.1	0.0062	7.9	0.0077	9.7
Level 3	0.171	0.0029	1.7	0.0068	3.9	0.0087	5.1	0.0114	6.7
Level 4	0.482	0.0053	1.1	0.0083	1.7	0.0136	2.8	0.0169	3.5
Level 5	0.728	0.0083	1.1	0.0114	1.6	0.0199	2.7	0.0244	3.4

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

12.2 Comparison

A comparison study was performed by analysing 196 serum samples (including 163 clinical sera and 28 normal sera) using the Optilite C4 kit and an alternative commercially available assay. Passing Bablok regression analysis generated the following results:

y = 0.98x – 0.01 g/L (y = Optilite; x = predicate analyser)

correlation coefficient r = 0.990 (calculated by linear regression)

A comparison study was performed by analysing 60 paired serum and EDTA plasma samples using the Optilite C4 assay. Passing Bablok regression analysis generated the following results:

y = 0.98x + 0.00 g/L (y = EDTA plasma; x = serum)

correlation coefficient r = 0.981 (calculated by linear regression)

A comparison study was performed by analysing 60 paired serum and Lithium Heparin plasma samples using the Optilite C4 assay. Passing Bablok regression analysis generated the following results:

y = 0.97x + 0.01 g/L (y = Lithium Heparin plasma; x = serum)

correlation coefficient r = 0.974 (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, 0.0064 g/L. The LoQ validation study was based on CLSI EP17-A *Protocols for Determination of Limits of Detection and Limits of 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 0.049 – 0.952 g/L using the 1+9 sample dilution has been demonstrated.

Regression equation: y = 0.9398x + 0.0226 (y = measured concentration, x = theoretical concentration); $r^2 = 0.9953$

12.5 Interference

A study was performed following CLSI EP7-A2: Interference Testing in Clinical Chemistry, Approved Guideline (CLSI Document EP7-A2). A serum sample close to the medical decision point and an abnormal serum sample were tested. No significant assay interference effects were observed when tested with bilirubin (200mg/L) or haemoglobin (5g/L). Intralipid and triglyceride showed signs of interference and lipaemic samples are known to interfere with this assay. Therefore lipaemic samples should not be analysed using this assay (see Section 10.2 for details).

No significant interference from commonly used therapeutic drugs is known. Further information is provided in literature (Ref 5).

12.6 Antigen excess

No antigen excess was observed up to a level of 5 times the top of the calibration curve at the standard 1+9 sample dilution. This is equivalent to 4.76 g/L.

13 BIBLIOGRAPHY

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