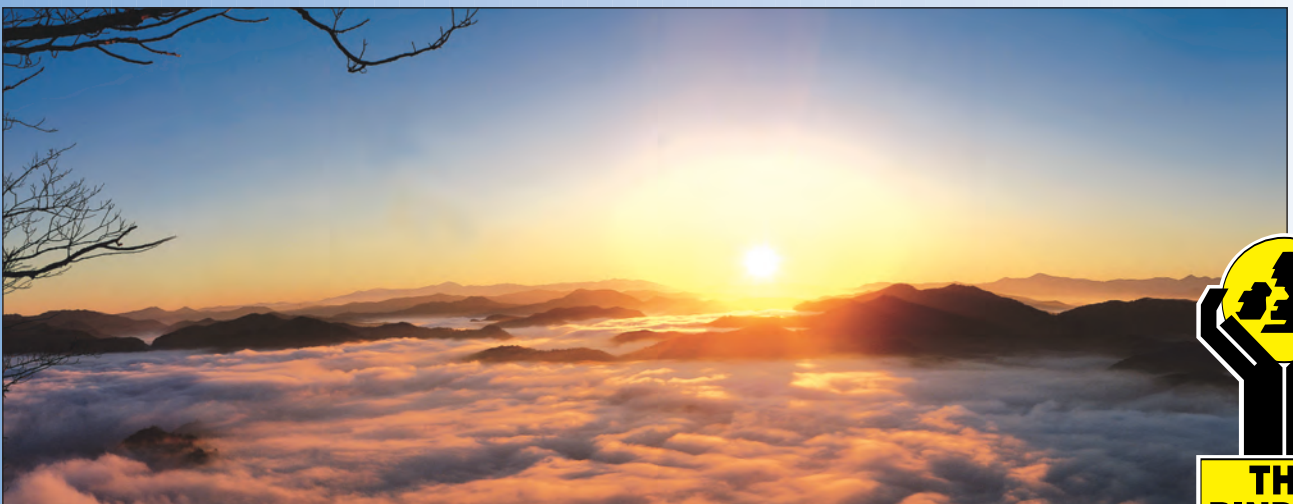


Assays for Investigation of Complement Function

Total Haemolytic Complement (THC) and Alternative Pathway Haemolytic Complement (APHC) kits are effective screening assays to aid in the identification of complement deficiency.

- Simple, inexpensive methodology
- Kit format to suit individual needs
- Functional assessment of total complement activity

SPECIALISTS IN HUMAN COMPLEMENT



The Classical Pathway

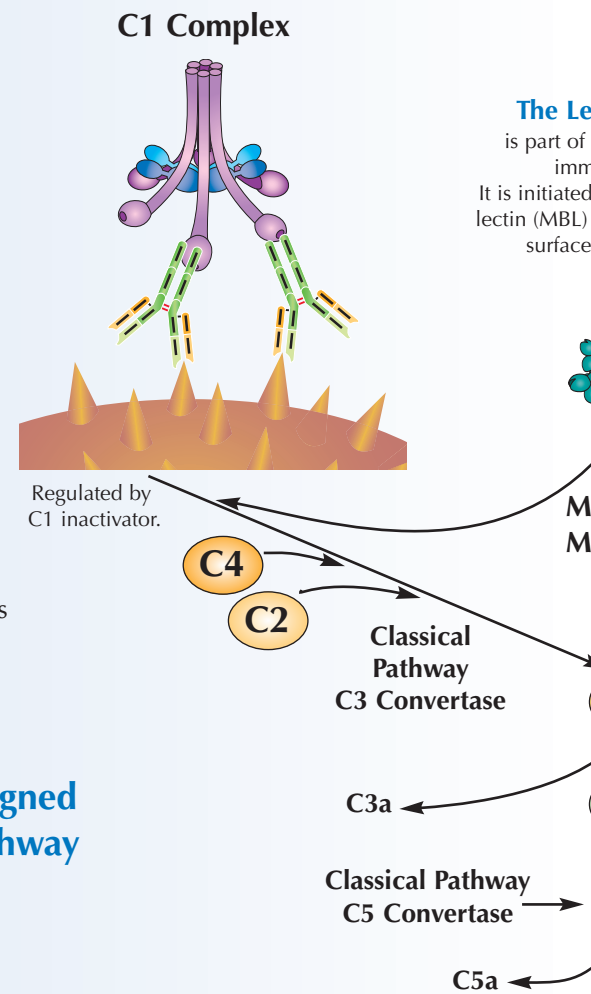
The **Classical Pathway** is part of the adaptive immune system.

In vivo the Classical Pathway is activated by the Fc portions of an immunoglobulin, binding to either an antigen on the cell wall of a micro-organism or a variety of other substances including endotoxins.

The formation of the C1 antigen-antibody complex triggers a cascade of reactions which activates complement components C4 and C2 leading to the formation of C3 convertase.

The activation of C3 then leads to the subsequent formation of C5 convertase and ultimately components C5, C6, C7, C8 and C9.

Components of C5b to C9 form the membrane attack complex which inserts into the lipid bilayer and lyses the cell.



The Total Haemolytic Complement kit is primarily designed to detect deficiencies of the Classical Complement Pathway and the terminal sequence (C3-C9) components.

ASSAY PRINCIPLE

Radial Immunodiffusion (RID) is a well established technique based on the binding of antigen and antibody to produce a visible precipitin ring in a gel. The Total Haemolytic Complement (THC) assay is an adaptation of the traditional RID assay. It uses the principle that sheep erythrocytes coated with anti-sheep erythrocyte antibody (haemolysin) will, in the presence of normal human serum, activate the classical complement pathway leading to haemolysis of the erythrocytes.

Functional Complement Assays

Erythrocytes coated with haemolysin are supported in agarose in the RID plate. Samples are added to the wells in the plate and incubated. This allows the complement components to diffuse through the gel prior to activation of the complement cascade at 37°C. A clear zone of haemolysis is produced around the well. The diameter of this zone is measured and can be used to calculate the haemolytic activity of the sample.

Functional complement assays are effective screening tools for complement deficiencies and the monitoring of complement activity in a model system.

The Alternative Pathway

Mannan-Binding Lectin Pathway

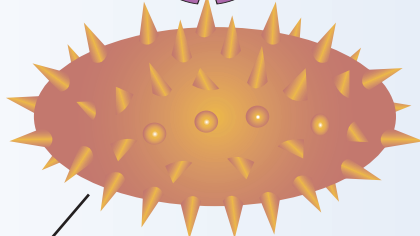
is part of the human innate immune system. It is triggered by mannans binding to microbial carbohydrates.



MBL-1
MBL-2

Activator surface
eg. bacterial cell wall

C3b



Factor B
Factor D

Positive feedback loop controlled by factor H and factor I.

Alternative Pathway
C3 Convertase

Properdin stabilises alternative pathway C3 convertase.

C3

C3b

C3a

Alternative Pathway
C5 Convertase

C5

C5b

C6

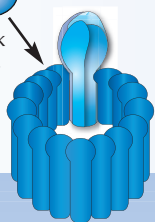
C7

C8

C9

MAC

MAC (membrane attack complex) forms a pore in the lipid bilayer allowing entry of ions and water into the cell. This results in a loss of osmotic gradient and cell lysis.



The Alternative Pathway forms part of the innate immune system. This pathway is triggered by components of bacterial endotoxins, yeast cell walls, aggregated immunoglobulins and snake venom.

The Alternative Pathway relies on the continuous low level hydrolysis of C3 to C3b.

C3b and antigen trigger the activation of Factor B in the presence of Factor D and leads to the formation of C3 convertase and subsequently C5 convertase.

The cascade then continues with the activation of components C5-C9 and the formation of the membrane attack complex.

The Alternative Pathway Haemolytic Complement kit is designed to measure the activity of the Alternative Complement Pathway.

ASSAY PRINCIPLE

The Alternative Pathway Haemolytic Complement (APHC) assay is an adaptation of the traditional RID assay, using the principle that chicken erythrocytes will bind C3b allowing the formation of C3 convertase. This activates the alternative complement pathway leading to the haemolysis of the erythrocytes.

- Simple methodology, no sample dilutions are necessary
- A 2 step incubation allows the formation of large, easy to read zones of haemolysis
- Results are interpreted easily without the need for expensive equipment
- Assays are supplied in kit format for ease of use
- 1, 2 or 3 plate kits are available to suit individual laboratory requirements

These assays that, used together, aid in the detection and monitoring of total complement activity.

Functional Complement Kits

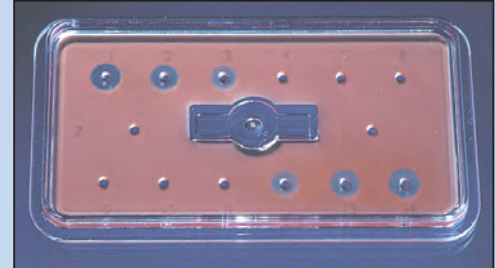
Ordering Information

DESCRIPTION	PACK	CODE
Total Haemolytic Complement kit	3 plate kit	RC001.3
	2 plate kit	RC001.2
	1 plate kit	RC001.1
Alternative Pathway Haemolytic Complement kit	3 plate kit	RC003.3
	1 plate kit	RC003.1
C1 Inactivator Functional activity kit	3 plate kit	RC002.3
C1 Inactivator Functional activity COMBI kit*	3 plate kit	RK019

* Combi kit contains 2 functional activity plates and 1 antigenic quantitation plate.

Kit Contents

- 1, 2 or 3 RID plates, 14 pre-cut wells per plate
- Calibrators
- Control
- Instruction leaflet



Quantitative Complement Kits

In addition to the functional complement kits, The Binding Site also provides a range of RID kits to quantify a wide selection of important complement cascade components and factors.

Kit Contents

- 3 RID plates, 14 pre-cut wells per plate
- Calibrators
- Control
- Sample diluent
- Gel sectioning blades
- Instruction leaflet and results table



Ordering Information

DESCRIPTION	PACK	CODE
C1 Inactivator - NL Range 45 to 450 mg/L	3 plate kit	RN019.3
C1q - NL Range 23 to 230 mg/L*	3 plate kit	RN020.3
C2 - NL Range 3.6 to 36 mg/L	3 plate kit	RN022.3
C3 - NL Range 155 to 1550 mg/L	3 plate kit	RN023.3
C4 - NL Range 58 to 580 mg/L	3 plate kit	RN025.3
C4 - Binding Protein - NL Range 50 to 500 mg/L	3 plate kit	RN026.3
C5 - NL Range 20 to 200 mg/L	3 plate kit	RN027.3
C6 - NL Range 12 to 120 mg/L	3 plate kit 1 plate kit	RN102.3 RN102.1
C7 - NL Range 22 to 110 mg/L	3 plate kit 1 plate kit	RN103.3 RN103.1
C8 - NL Range 20 to 200 mg/L	3 plate kit 1 plate kit	RN089.3 RN089.1
C9 - NL Range 50 to 500 mg/L	3 plate kit 1 plate kit	RN028.3 RN028.1
Factor B - NL Range 45 to 450 mg/L	3 plate kit	RN029.3
Factor H (β 1H) - NL Range 70 to 700 mg/L	3 plate kit 1 plate kit	RN030.3 RN030.1
Factor I - NL Range 7 to 70 mg/L	1 plate kit	RN031.1

* Diluted sample applied - assay range may be extended using undiluted sample.



MKG274.1

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