

INSTRUCTION FOR USE

WIESLAB® Complement system

Alternative pathway

Enzyme immunoassay for assessment of
Complement functional activity

Break apart microtitration strips (12x8) 96 wells

Store the kit at +2-8° C

Store the positive control at -20° C

FOR RESEARCH USE ONLY
IN THE US & CANADA



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REF

COMPL AP330

IVD

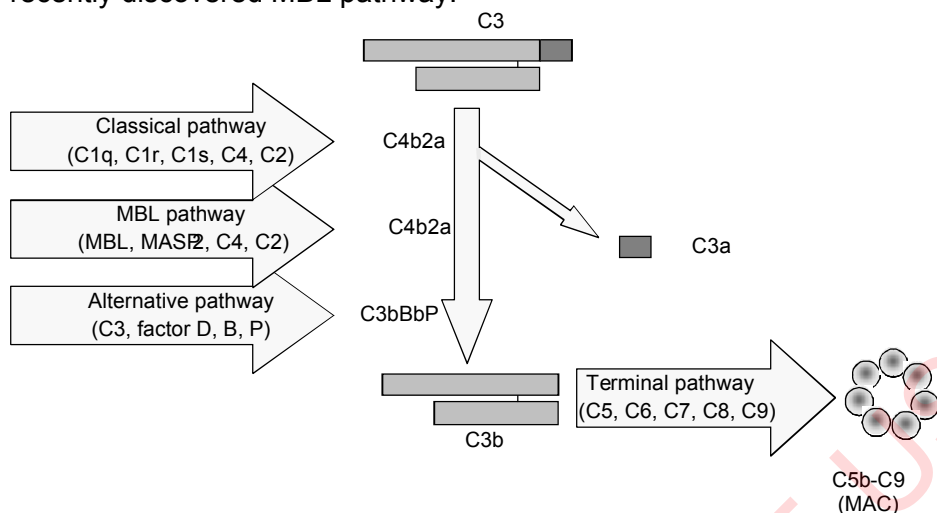


INTENDED USE

The Wieslab® Complement system Alternative pathway kit is an enzyme immunoassay for the qualitative determination of functional alternative complement pathway in human serum.
FOR *IN VITRO* DIAGNOSTIC USE.

Summary and explanation

The complement system plays an essential role in chronic, autoimmune and infectious disease. There are three pathways of complement activation (fig. 1), namely the classical, the alternative and the recently discovered MBL pathway.



Impaired complement activity causes humans to become susceptible to repetitive fulminant or severe infections and may contribute to development of autoimmune disease. Inappropriate activation of complement contributes to chronic inflammation and tissue injury.

Principle of the Wieslab® Complement assay

The Wieslab® Complement assay combines principles of the hemolytic assay for complement activation with the use of labeled antibodies specific for neoantigen produced as a result of complement activation. The amount of neoantigen generated is proportional to the functional activity of complement pathways.

In the Complement system AP kit the wells of the microtitre strips are coated with specific activators of the alternative pathway. Patient serum is diluted in diluent containing specific blocker to ensure that only the alternative pathway is activated. During the incubation of the diluted patient serum in the wells, complement is activated by the specific coating.

The wells are then washed and C5b-9 is detected with a specific alkaline phosphatase labelled antibody to the neoantigen expressed during MAC formation.

After a further washing step, detection of specific antibodies is obtained by incubation with alkaline phosphatase substrate solution. The amount of complement activation correlates with the colour intensity and is measured in terms of absorbance (optical density (OD)).

Warnings and precautions

- FOR IN VITRO DIAGNOSTIC USE.
- The human serum components used in the preparation of the controls in the kit have been tested for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen by FDA approved methods and found negative. Because no test methods can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
- The Centers for Disease Control and Prevention and National Institutes of Health recommended that potentially infectious agents be handled at the Biosafety Level 2.
- All solutions contain ProClin 300 as a preservative. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing ProClin may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.
- Material safety data sheets for all hazardous components contained in this kit are available on request from Svar Life Science.

Wash Solution (30x Conc.)**WARNING**

Contains: Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl- 2H-isothiazol-3-one [EC no. 220-239-6] (3:1)

H317 May cause an allergic skin reaction.
 H412 Harmful to aquatic life with long lasting effects.
 P261 Avoid breathing spray.
 P273 Avoid release to the environment.
 P280 Wear protective gloves.
 P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

Dilution Buffer AP, Conjugate Solution, Negative Control, Substrate pNPP

EUH208 Contains "Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazolin-3-one [EC no. 220-239-6] (3:1)" May produce an allergic reaction.
 EUH210: Safety data sheet available on request.

Specimen collection

Blood samples are to be collected using aseptic venipuncture technique and serum obtained using standard procedures. A minimum of 5 mL of whole blood is recommended. Allow blood to clot in serum tubes, for 60-65 minutes at room temperature (20-25° C). Centrifuge blood samples and transfer cell-free serum to a clean tube. **Sera must be handled properly to prevent *in vitro* complement activation.** Sera should be frozen at -70° C or lower in tightly sealed tubes for extended storage or for transport on dry ice. Samples should not be frozen and thawed more than once.

Avoid using sera which are icteric, lipemic and hemolyzed.

Heat-inactivated sera cannot be used. Plasma can not be used.

The NCCLS provides recommendations for storing blood specimens, (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18A, 1990).

Kit components and storage of reagents

- One frame with colourless break-apart wells (12x8) sealed in a foil pack with a desiccation sachet. The wells are coated with LPS.
- 35 mL Diluent AP (Dil AP), labelled red.
- 13 mL conjugate containing alkaline phosphatase-labelled antibodies to C5b-9 (blue colour).
- 13 mL Substrate solution ready to use.
- 30 mL wash solution 30x concentrated.
- 0.2 mL negative control (NC) containing human serum. (to be diluted as for a patient serum sample).
- 0.2 mL positive control (PC) containing freeze-dried human serum, see "Reconstitution of positive control", below.

All reagents in the kit are ready for use except washing solution and controls. The reagents should be stored at 2-8° C except the positive control. **The positive control must be stored at -20°C.**

Materials or equipment required but not provided

- Microplate reader with filter 405 nm.
- Precision pipettes with disposable tips.
- Washer for strips, absorbent tissue, tubes and a timer.

PROCEDURE

Remove only the number of wells needed for testing, resealing the aluminium package carefully. Let all solutions equilibrate to room temperature (20-25° C) before analysis.

Preparation of washing solution

In case salt crystals are observed in the vial with concentrated wash solution, place the vial at 37°C water bath until the crystals have dissolved before dilution of wash solution. Dilute 30 mL of the 30x concentrated wash solution in 870 mL distilled water. When stored at 2-8° C, the diluted wash solution is stable until the date of expiration of the kit.

Reconstitution of positive control

Gently tap down all lyophilized material to the bottom of the vial and remove the cap. Immediately add 200 µl of distilled water directly to the lyophilized material. Replace the cap. Allow the vial to stand on ice for 5 minutes and then gently shake or vortex occasionally until completely dissolved. Dilute the reconstituted control in the same way as a patient serum sample. The reconstituted control can be stored for up to 4 hours prior to use if kept at 2-8° C or on ice. It can be frozen at -70° C and thawed once.

Serum

Partially thaw frozen sera by briefly placing in a 37° C water bath with gentle mixing. After partially thawing immediately place the tubes in an ice bath and leave on ice until completely thawed. Mix briefly on a vortex mixer.

Dilution of serum

Dilute the serum 1/18 with Diluent AP, red label, (340 µL Diluent + 20 µL serum). The diluted serum can be left at room temperature for a maximum of 60 minutes before analysis.

Incubation of samples

Pipet 100 µL/well in duplicate of Diluent (Dil AP) as a blank, positive control (PC), negative control (NC) and diluted patient's serum (P) for each pathway according to the diagram. Incubate for 60-70 minutes at +37° C with lid.

Alternative Pathway

	1	2	3
A	Dil AP	P2	
B	Dil AP	P2	
C	PC	etc	
D	PC		
E	NC		
F	NC		
G	P1		
H	P1		

After serum incubation

Empty the wells and wash 3 times with 300 µL washing solution, filling and emptying the wells each time. After the last wash, empty the wells by tapping the strip on an absorbent tissue.

Adding conjugate

Add 100 µL conjugate to each well. Incubate for 30 minutes at room temperature (+20-25° C).

After conjugate incubation

Wash 3 times as before.

Adding substrate solution

Add 100 µL substrate solution to each well, incubate for 30 minutes at room temperature (+20-25° C). Read the absorbance at 405 nm on a microplate reader. (5 mM EDTA can be used as stop solution, 100 µl/well. Read the absorbance of the wells within 60 minutes.)

Calculation of result

Subtract the absorbance of the Blank (Diluent) from the NC, PC and the samples. The absorbance of the positive control should be >1 and the negative control absorbance < 0.2.

The negative and positive controls can be used in a semiquantitative way to calculate complement activity. Calculate the mean OD405nm values for the sample, PC and NC and calculate the % complement activity as follows: $(\text{Sample}-\text{NC})/(\text{PC}-\text{NC}) \times 100$. The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cut-off. It is recommended that each laboratory establish its own reference level and cutoff value for deficiencies.

A negative result i.e. deficiency, should always be verified by testing a new sample to ensure that no in vitro complement activation has taken place.

Limitations

The individual patient's complement level cannot be used as a measure of disease severity, as it may vary from patient to patient. Thus, it is difficult to obtain an absolute standardisation of results.

The test should not be relied upon as the sole basis of decisions on clinical therapy, but should be used in combination with clinical symptoms and the results of other available tests. Therapy should not be started on basis of the complement assay result. Initiation or changes in treatment should not be based on changes in complement levels alone, but rather on careful clinical observation.

Expected results

In vitro activation of the complement sequence leads to the consumption of complement components which, in turn, leads to a decrease in their concentration. Thus, the determination of complement proteins or complement activity is used to indicate whether the complement system has been activated by an immunologic and/or pathogenic mechanism. Both functional and immunochemical complement measurements are used to evaluate patients when a complement-activating disease is suspected or an inherited deficiency is possible. The level of complement activity evaluated by functional assays such as Wieslab® Complement kit takes into account the rate of synthesis, degradation, and consumption of the components and provides a measure of the integrity of the pathways as opposed to immunochemical methods, which specifically measure the concentration of various complement components. When decreased levels of complement components or complement function are found, a deficiency or an ongoing, immunologic process, leading to increased breakdown of components and depression of complement levels is considered by clinicians. Increased complement levels are usually a nonspecific expression of an acute phase response.

The Wieslab® Complement system AP can be helpful for detection of complement deficiencies related to the Alternative Pathways as shown in the table below: A more complete and in-depth functional assessment of all three complement pathways may be achieved using Wieslab® Complement system Screen.

Classical pathway	MBL pathway	Alternative pathway	Possible deficiency
Positive	Positive	Positive	None
Negative	Positive	Positive	C1q, C1r, C1s
Positive	Positive	Negative	Properdin, Factor B, D
Positive	Negative	Positive	MBL, MASP2
Negative	Negative	Negative	C3, C5, C6, C7, C8, C9
Negative	Negative	Positive	C4, C2 or combination

Performance characteristics

120 sera from blood donors were tested in the AP assay and the normal reference range was calculated. The values were expressed in % of the positive control. See Figure 1 and Table 1. In the study no blood donor was below 10 %.

Figure 1 AP assay.

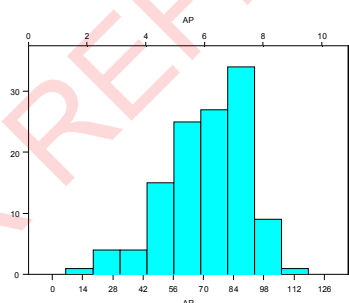


Table 1.

	n	Mean (%)	±2SD (%)	Median (%)
Alternative pathway	120	71	30-113	73

Table 2

Sera with known complement deficiencies were tested in the assay and the following results were obtained. All deficient sera were detected in the assay and gave values below 5 %.

Deficiency	C3	C5	C6	C7	C8	C9	P	H	I
Number of patients	1	2	1	2	2	1	9	1	2
Number of deficient sera detected	1	2	1	2	2	1	9	1	2

Table 3. Inter-assay precision was determined by testing three samples in duplicate. Results were obtained for six different runs.

Sample	Mean value %	SD	CV %
P1	48	5.1	11
P2	89	8.0	9
P3	16	3.1	20

Table 4. Intra-assay precision was determined by testing one sample in 40 wells.

Assay	Mean value %	SD	CV %
AP	83	5.7	7

Troubleshooting

PROBLEM	POSSIBLE CAUSES	SOLUTION
Control values out of range	Incorrect temperature, timing or pipeting, reagents not mixed	Check that the time and temperature was correct. Repeat test.
	Cross contamination of controls	Pipette carefully.
	Optical pathway not clean.	Check for dirt or air-bubbles in the wells. Wipe plate bottom and reread.
	Positive control not properly dissolved.	Check the positive control dissolve a new.
All test results negative	One or more reagents not added, or added in wrong sequence.	Recheck procedure. Check for unused reagents. Repeat test.
	Antigen coated plate inactive.	Check for obvious moisture in unused wells. Wipe plate bottom and reread.
	Serum inactive.	Dilute new samples.
All test results yellow.	Contaminated buffers or reagents.	Check all solutions for turbidity.
	Washing solution contaminated.	Use clean container. Check quality of water used to prepare solution.
	Improper dilution of serum.	Repeat test.
Poor precision.	Pipette delivery CV >5% or samples not mixed.	Check calibration of pipette. Use reproducible technique. Avoid airbubbles in pipette tip.
	Serum or reagents not mixed sufficiently or not equilibrated to room temperature.	Mix all reagents gently but thoroughly and equilibrate to room temperature.
	Reagent addition taking too long, inconsistency in timing intervals.	Develop consistent uniform technique and use multi-tip device or autodispenser to decrease time.
	Optical pathway not clean.	Check for airbubbles in the wells. Wipe plate bottom and reread.

PROBLEM	POSSIBLE CAUSES	SOLUTION
	Washing not consistent, trapped bubbles, washing solution left in the wells.	Check that all wells are filled and aspirated uniformly. Dispense liquid above level of reagent in the well. After last wash, empty the wells by tapping the strip on an absorbent tissue.

References:

- Walport M, Complement (First of two parts) N Engl J Med 2001, 344, 1058-1066.
- Walport M, Complement (Second of two parts) N Engl J Med 2001, 344, 1140-1144.
- Roos A, Bouwman L, Munoz J et al., Functional characterization of the lectin pathway of complement in human serum. Mol Immunol 2003, 39, 655-668.
- Nordin Fredriksson G, Truedsson L, Sjöholm A. New procedure for detection of complement deficiency by ELISA. J Imm Meth 1993, 166, 263-270.
- M.A. Seelen et al, Functional analysis of the classical, alternative and MBL pathways of the complement system: standardization and validation of a simple ELISA. J Imm Meth 2005, 296, 187-198.

Explanation of symbols. L'explication de symboles. La explicación de símbolos. Erklärung der Symbole. La spiegazione di simboli. Explicação dos símbolos. Forklaring til symboler. Symbolforklaring. Förklaringar till symboler.

	Batch code. Numéro de lot. Número de lote. Chargen-Nummer. Numero di lotto. Código do lote. Partnummer. Lot nummer. Satsnummer.
	Catalogue number. Référence catalogue. Número de catálogo. Katalog-Nummer. Numero di catalogo. Número catalogo. Katalognummer.
	Use-by date. Date de péremption. Fecha de caducidad. Verfallsdatum. . La data di scadenza. Prazo de validade. Udløbsdato. Utløpsdato. Använd före.
	Temperature limit. Seuils de températures. Rango de temperature. Temperaturbereich. Limitazioni di temperatura. Limite de temperatura. Opbevaringstemperatur. Oppbevares ved. Förvaringstemperatur.
	Biological risk. Risque biologique. Riesgo biológico. Biologische Gefährdung. Rishio biologico. Risco biológico. Biologisk risk.
	Consult instructions for use. Lire le mode d'emploi. Consulte las instrucciones de uso. Gebrauchsanweisung beachten. Leggere le istruzioni per l'uso. Consultar as instruções de utilização. Se brugsanvisning. Se bruksanvisningen. Läs instruktionsmanualen.
	In vitro diagnostic medical device. Dispositif médical de diagnostic in vitro. Producto sanitario para diagnóstico in vitro. In-vitro-Diagnostikum. Dispositivo medico-diagnostico in vitro. Dispositivos médicos para diagnóstico <i>in vitro</i> . In Vitro medisinsk diagnoseutstyr. In vitro diagnostik medicinsk utrustning.
	Warning. Attention. Atención. Achtung. Attenzione. Atenção. Advarsel. Advarsel. Varning.
	Manufacturer. Fabricant. Fabricante. Hersteller. Produttore. Fabricante. Fabrikant. Producent. Produsent. Tillverkare.
	Contains sufficient for 96 tests. Contenu suffisant pour 96 tests. Contenido suficiente para 96 pruebas. Inhalt ausreichend für 96 Tests. Contenuto sufficiente per 96 test. Indeholder tilstrækkelig for 96 test. Inneholder tilstrekkelig for 96 test. Innehåller tillräckligt för 96 test.
	Conformity to 98/79/EC on In Vitro Diagnostic Medical Device Directive. Conformément à la directive européenne 98/79/CE relative aux dispositifs médicaux de diagnostic in vitro. La conformidad con la Directiva 98/79/CE sobre productos sanitarios para diagnóstico in vitro. Konform mit Richtlinie 98/79/EG zu In-vitro-Diagnostika. Conformità alla direttiva 98/79/CE relativa ai dispositivi medico-diagnostici in vitro. Medicinsk utstyr til in vitro-diagnostik, i overensstemmelse med Europa-Parlamentets og Rådets direktiv 98/79/EF. Medisinsk utstyr i samsvar med EU in vitro diagnostic directive 98/79/EF. Överensstämmer med direktiv 98/79/EG för medicintekniska produkter.

Ag	Antigen. Antigène. Antigeno, Antigen. L'antigene. Antígeno. Antigen. Antigen. Antigen.
DIL	Diluent. Diluant. Diluyente. Probenverdünnungspuffer. Il diluente. Diluente. Diluent. Fortynning. Spädningsbuffert.
CONJ	Conjugate. Conjugué. Conjugado. Konjugat. Conjugato. Conjugado. Konjugat. Konjugat.
BUF WASH 30X	Wash solution 30x conc. Solution lavage conc. 30x. Solución de lavado conc. 30x. Waschpuffer 30x konz. Soluzione di lavaggio 30x conc. Solução de lavagem conc. 30 vezes. Vaskebuffert 30x konc. Vaskeløsning 30x kons. Tvättbuffert 30x konc.
SUBS pNPP	Substrate pNPP. pNPP Substrat. Sustrato pNPP. Substrat pNPP. Substrato pNPP. Substrato pNPP. Substrato pNPP. Substrat pNPP.
CONTROL -	Negative control. Contrôle négatif. Control negativo. Negativkontrolle. Controllo negativo. Controllo negativo. Negativ control. Negativ kontroll. Negativ kontroll.
CONTROL + LYO	Lyophilized positive control. Contrôle positif lyophilisé. Control positivo liofilizado. Lyophilisierte Positivkontrolle. Controllo positivo liofilizzato. Controllo positivo liofilizado. Frysetørret positiv kontroll. Lyofilisert positiv kontroll. Frystorkad positiv kontroll.
CH REP	Swiss Representative. Représentant Suisse. Representante suizo. Schweizer Vertreter. Rappresentante svizzero. Representante da Suica. Schweizisk repræsentant. Sveitsisk representant. Schweizisk representant

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