

INSTRUCTIONS FOR USE

WIESLAB® Complement System Screen

Enzyme immunoassay for assessment of
Complement functional activity

Break apart microtitration strips (4x8x3) 96 wells
Store the kit at +2-8° C
Store the positive control at -20° C

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**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES**



COMPL 300 RUO

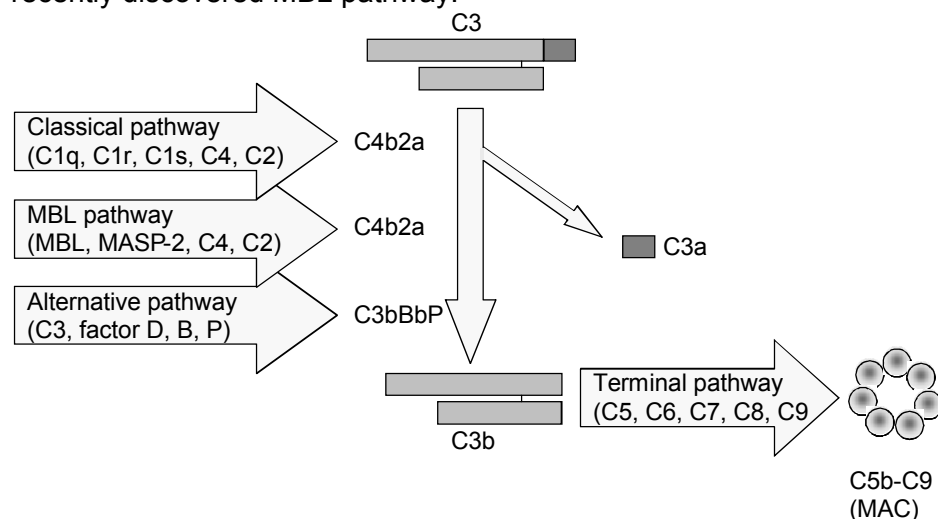


PURPOSE OF RESEARCH PRODUCT

The Wieslab® Complement system Screen kit is an enzyme immunoassay for the qualitative determination of functional classical, MBL and alternative complement pathways in human serum, the result shall not be used for clinical diagnosis or patient management.

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 labelled 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.

The wells of the microtitre strips are coated with specific activators of the classical, or the MBL, or the alternative pathway. Subject serum is diluted in diluent containing specific blocker to ensure that only the respective pathway is activated. During the incubation of the diluted subject 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 Research Use Only. Not for use in diagnostic procedures.**

- 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 CP, Dilution Buffer MP, 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 can not 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 break apart wells (4x8x3) sealed in a foil pack with a desiccation sachet. 4 blue coloured strips for classical pathway (CP), coated with human IgM. 4 green coloured strips for MBL pathway (MP), coated with mannan. 4 colourless strips for alternative pathway (AP), coated with LPS.
- 10 ml Diluent CP (Dil CP), labelled blue.
- 10 ml Diluent MP (Dil MP), labelled green.
- 10 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 subject 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 should 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 subject 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

Classical Pathway (CP): Dilute the serum 1/101 with Diluent CP, blue label, (500 µl Diluent + 5 µl serum). The diluted serum can be left at room temperature for a maximum of 60 minutes before analysis.

MBL Pathway (MP): Dilute the serum 1/101 with Diluent MP, green label, (500 µl Diluent + 5 µl serum). The diluted serum must be left at room temperature for >15 minutes before analysis, but not more than 60 minutes.

Alternative Pathway (AP): 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) as a blank, positive control (PC), negative control (NC) and diluted subject's serum (P) for each pathway according to the diagram. Incubate for 60-70 minutes at +37° C with lid.

Please note that no incubation should be performed under CO₂ atmosphere. If a CO₂ cabinet is used, make sure that the CO₂ supply is disconnected/off.

	Classical Pathway				MBL Pathway				Alternative Pathway			
	1	2	3	4	5	6	7	8	9	10	11	12
A	Dil CP	P2			Dil MP	P2			Dil AP	P2		
B	Dil CP	P2			Dil MP	P2			Dil AP	P2		
C	PC	etc			PC	etc			PC	etc		
D	PC				PC				PC			
E	NC				NC				NC			
F	NC				NC				NC			
G	P1				P1				P1			
H	P1				P1				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) (for each pathway) 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 OD_{405nm} values for the sample, PC and NC and calculate the % complement activity as follows: (Sample-NC)/(PC-NC)x100. 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.

Subject results

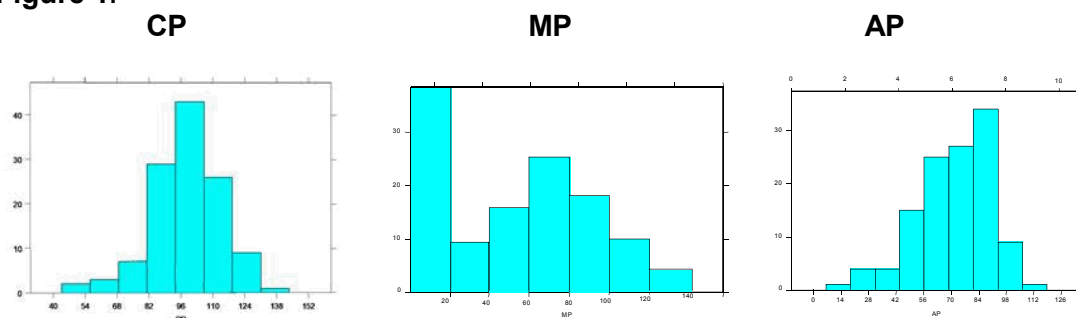
In vitro activation of the complement sequence leads to the consumption of complement components which, in turn, leads to a decrease in their concentration. Thus, the determination of complement proteins or complement activity is used to indicate whether the complement system has been activated by an 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 combination of the three Wieslab® assays for complement can be helpful for detection of complement deficiencies as shown in the table below:

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 three assays and the normal reference range was calculated. The values were expressed in % of the positive control. See Figure 1 and Table 1. In the CP assay no blood donor was below 40 %. In the MP assay 23 samples were below 10 % and they had MBL values (established in a separate assay) below 500 ng/ml and in the AP assay no blood donor was below 10 %. Please note that true deficient MP, i.e. an activity of <10%, may be found in a normal population at a frequency of 20-30%.

Figure 1.**Table 1.**

	n	Mean (%)	$\pm 2SD$ (%)	Median (%)
Classical pathway	120	99	69-129	100
MBL pathway	120	49	0-125	56
Alternative pathway	120	71	30-113	73

Table 2

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

Deficiency	C1q	C2	C3	C4	C5	C6	C7	C8	C9	P	H	I
Number of subjects	3	11	1	1	2	1	2	2	1	9	1	2
Number of deficient sera detected	3	11	1	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 %
CP P1	98	4.3	4
CP P2	92	3.9	4
CP P3	21	1.7	8
MP P1	91	3.3	4
MP P2	37	4.0	11
MP P3	16	2.3	15
AP P1	48	5.1	11
AP P2	89	8.0	9
AP P3	16	3.1	20

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

Assay	Mean value %	SD	CV %
CP	85	2.9	3
MP	74	3.9	5
AP	83	5.7	7










Troubleshooting

Problem	Possible causes	Solution
Control values out of range	Incorrect temperature, timing or pipetting, 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.
	Mixup of plates or reagents from another pathway. Improper dilution.	Repeat test.
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.
	Mixup of plates or reagents from another pathway.	Repeat test.
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.
	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.

Explanation of symbols.

	Use-by date.
	Biological risks.
	Temperature limit.
	Manufacturer.
	Batch code.
	Catalogue number.
	Consult instructions for use.
	Warning.
	Contains sufficient for 96 tests.

Ag	Antigen.
DIL	Diluent.
CONJ	Conjugate.
BUF WASH 30X	Wash solution 30x conc.
SUBS pNPP	Substrate pNPP.
CONTROL -	Negative control.
CONTROL + LYO	Lyophilized positive control.

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