

Product Information

Complement C4 deficient serum from guinea pig

Catalog Number **C1038**

Storage Temperature $-70\text{ }^{\circ}\text{C}$

Product Description

Complement C4 Deficient Serum is isolated from C4 deficient guinea pigs. The serum is C4 deficient as judged by a highly sensitive hemolytic assay and an Ouchterlony immunodiffusion method.

The product is supplied as a $0.2\text{ }\mu\text{m}$ filtered solution, pH ~ 7.9 .

The complement system is a complex cascade involving proteolytic cleavage of serum glycoproteins often activated by cell receptors. This cascade ultimately results in induction of the inflammatory response, phagocyte chemotaxis and opsonization, and cell lysis.

Complement factors C3a, C5a, and C4 can induce vasodilatation, increased capillary permeability, and expression of leukocyte adhesion molecules. Complement C3a and C4b are opsonins that bridge phagocytes to microorganisms and promote phagocyte chemotaxis.

The C4H50 unit is used to express the complement C4 hemolytic activity using guinea pig C4 deficient serum. One C4H50 unit is defined as the amount of complement standard serum or sample containing complement C4 to yield 50% lysis of 3×10^7 antibody sensitized sheep erythrocytes when incubated in the presence of the recommended volume of C4 deficient serum for 30 minutes at $37\text{ }^{\circ}\text{C}$ in a final volume of $500\text{ }\mu\text{l}$.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at $-70\text{ }^{\circ}\text{C}$ is recommended. Repeated freezing and thawing is **not** recommended.

Procedure

The following procedure is used for the determination of C4 activity. The assay should be performed in an ice bath, except where otherwise indicated.

1. Prepare 8 precooled assay tubes labeled "A" through "H" and 2 precooled control tubes labeled "Spontaneous Lysis" and "100% Lysis".
2. Thaw the C4 deficient serum in a $37\text{ }^{\circ}\text{C}$ water bath. Do not thaw at $4\text{ }^{\circ}\text{C}$ or at room temperature.
3. Place the thawed C4 deficient serum into an ice bath immediately and pipette the recommended volume (v , see lot-specific CofA) into the precooled assay tubes.
4. Dilute the complement C4 to a concentration in the range of $100\text{--}500\text{ ng/ml}$ with ice cold gelatin veronal buffer (GVB²⁺, Catalog Number G6514). If human whole serum is used, dilute 200 to 3,000-fold with ice cold GVB²⁺.
Note: The above serum dilution range is a suggestion only. Due to variability in sera, the actual serum dilution required should be determined by the investigator.
5. Prepare a suspension of 1.5×10^8 cells/ml of antibody sensitized sheep erythrocytes in GVB²⁺. For a procedure to prepare antibody sensitized sheep erythrocytes, please visit sigma-aldrich.com/complement.
6. Pipette the diluted complement C4 or human whole serum, antibody sensitized sheep erythrocytes, GVB²⁺, and distilled water into the assay tubes according to Table 1.
7. Incubate all tubes in a $37\text{ }^{\circ}\text{C}$ water bath with shaking for 30 minutes.
8. Add 1.0 ml of ice cold GVB²⁺ to each tube immediately after incubation.

9. Centrifuge the tubes at 2,000 rpm at 2–8 °C for 10 minutes.
10. Read the absorbance of the supernatant of each tube at 412 nm.
11. Calculate the hemolytic activity for C4 as follows:
 - a. Subtract the OD_{412 nm} of the "Spontaneous Lysis" solution from the OD_{412 nm} of each assay solution (A, B, . . . , H) and from the OD_{412 nm} of the "100% Lysis" solution. These values are represented as OD'₄₁₂. The OD'_{412 nm} of assay tube "A" represents the background activity. Note: Background activity should be determined every time for an assay with complement C4 deficient serum.
 - b. Calculate the value of y for each assay solution:

$$y = \frac{\text{OD}'_{412} \text{ of assay solution (A,B...H)}}{\text{OD}'_{412} \text{ of "100\% lysis" solution}}$$
 - c. Calculate the value of y/(1-y) for each assay solution (A, B, . . . , H).
 - d. Plot the value of y/(1-y) against the corresponding volume of human whole serum or complement C4 used in each assay solution on a sheet of 2 × 3 cycle log-log graph paper.
 - e. Determine the amount of human whole serum or complement C4 which gives 50% lysis (i.e., y/(1-y) = 1). This value corresponds to one C4H50 unit. The hemolytic titer is calculated as the reciprocal of the dilution, which gives 50% lysis (i.e., the amount of C4H50 units/ml standard serum or sample).

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Table 1.

The volumes indicated are an example only. Adjust the volumes of the C4-containing sample and GVB²⁺ as needed, keeping the total volume of the reaction mixture at 500 µl.

Assay Tubes	C4 deficient serum (µl)	Diluted human whole serum or purified C4* (µl)	Antibody Sensitized Sheep Erythrocytes (1.5 × 10 ⁸ cells/ml) (µl)	GVB ²⁺ (µl)	Distilled water (µl)
A**	v	–	200	300–v	–
B	v	5	200	295–v	–
C	v	10	200	290–v	–
D	v	20	200	280–v	–
E	v	30	200	270–v	–
F	v	40	200	260–v	–
G	v	50	200	250–v	–
H	v	60	200	240–v	–
Control Tubes					
100% Lysis	–	–	200	–	300
Spontaneous Lysis	–	–	200	300	–

* Either dilute human whole serum or purified complement C4 can be added to the reaction mixture to restore C4 activity.

** The OD'_{412 nm} of assay tube "A" represents the background activity.

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